FORM PTO-1390 (REV 12-97) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: P-1459(0)

U.S. APPLICATION NO. (If known, see 37 CFR 1 5): 09/142557

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA97/00172	03/12/97	03/14/96 .

TITLE OF INVENTION: METHODS FOR CELL MOBILIZATION USING IN VIVO TREATMENT WITH HYALURONAN (HA)

APPLICANT(S) FOR DO/EO/US: LINDA MAY PILARSKI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/CA97/00172	P-1459(O)

17. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Search Report has been prepared by the EPO or JPO **$930.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$1070.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$1,070.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	71 - 20 =	51	x **$22.00**	$1,122.00	
Independent claims	27 - 3 =	24	x **$82.00**	$1,968.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$4,560.00	
				Amount to be refunded:	$
				charged:	$

a. [X] A check in the amount of $4,560.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [ ] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. _____. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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/SIGNATURE

IVOR M. HUGHES
NAME

27,759
REGISTRATION NUMBER

IN THE UNITED STATES PATENT OFFICE

Application Serial No. (To be assigned) Our Ref. : P-1459(O)
Entry into National Phase from PCT/CA97/00172

Applicants: Hyal Pharmaceutical Corporation

Attorney : Ivor M. Hughes
Suite 200
175 Commerce
Valley Drive West
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L3T 7P6

Title: METHODS FOR CELL MOBILIZATION USING IN VIVO TREATMENT WITH HYALURONAN

Inventor: Linda May Pilarski

September 10, 1998

The Commissioner of Patents
UNITED STATES PATENT OFFICE
2011 South Clark Place
Crystal Plaza 2, Room 1B03
Arlington, Virginia 22202 U.S.A.

Dear Sir:

PRELIMINARY AMENDMENT

Preliminary to the examination of this application and before of the fees, applicants respectfully request that the following be entered.

Preliminary Amendment Not used to calculated claim fee because It addresses original claims as filed & not the ones

MS

Please cancel claims 1 to 93 and 95 to 100.

Please add the following new claims 101 to 170.

od of treating a patient for the same purposes as recombinant GM- is used, the method comprising administering an effective amount yaluronic acid selected from the group consisting of hyaluronic acid

and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons.

102. A method of treating a patient for the same purposes as recombinant erythropoietin is used, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons.

103. A method for enhancing the stimulation of cells production/release from the bone marrow and other tissue sites into the blood, the cells being selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, said method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight of less than 750,000 daltons.

104. A method of treating a patient by enhancing the stimulation and activation of stromal cells, comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight of less than 750,000 daltons.

105. A method of treating a patient by releasing cancer cells from bone marrow and other tissues into the blood, comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to an individual, the molecular weight of the form of hyaluronic acid being less than 750,000 daltons.

106. The method of Claim 101, 102, 103, 104, or 105 wherein the form of hyaluronic acid comprises at least about 1.5mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

107. The method of Claim 101, 102, 103, 104, or 105 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount and said form of hyaluronic acid is sodium hyaluronate.

108. The method of Claim 101, 102, 103, 104 or 105 wherein the form of hyaluronic acid is at least about 12 mg/kg of patient body weight and said form of hyaluronic acid is sodium hyaluronate.

109. The method of Claim 107 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

110. The method of Claim 108 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

111. A method for the administration to a human of an effective amount of a form of hyaluronic acid comprising an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for enhancing, stimulating and releasing hematopoietic cells and dendritic-type cells from the bone marrow and other tissues into the blood.

112. A method of treatment for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for stimulating and activating stromal cells.

113. A method of treatment for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for releasing cancer cells from the bone marrow and other tissues into the blood.

114. The method of Claim 111, 112 or 113 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

115. The method of Claim 111, 112 or 113 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount.

116. A method of treating a patient for enhancing the stimulation of cells production/release from the bone marrow and other tissues of cells selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, comprising administering a plurality of amounts of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient at predetermined intervals, at least one of such dosages being in an amount suitable to stimulate the production/release of the cells from the bone marrow and other tissues into the blood.

117. The method of Claim 116 wherein the interval between dosages is a week.

118. The method of Claim 116 or 117 wherein at least one of the amounts is a priming dosage for the patient and the form of hyaluronic acid is sodium hyaluronate.

119. The method of Claim 116 or 117 wherein the form of hyaluronic acid is sodium hyaluronate.

120. The method of Claim 119 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

121. The method of Claim 118 wherein one of the amounts is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

122. The method of Claim 116 or 117 wherein one of the dosages is a priming dosage in the amount of less than about 3 mg/kg of patient body weight.

123. The method of Claim 119 wherein one of the dosages is a priming dosage in the amount of less than about 3 mg/kg of patient body weight.

124. A method of treating a patient for mobilizing hematopoietic cells from bone marrow and other tissues in a human into the blood of the human, the

method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient.

125. A method of treating a patient for mobilizing stem cells from bone marrow in a human into the circulation system of the human, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to the patient.

126. A method of generating stem cells for transplantation comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to an individual and subsequently harvesting the cells to be transplanted from the peripheral blood.

127. A method of treating a patient for immunosuppression caused by chemotherapy comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient who has undergone chemotherapy.

128. A method of a treating a patient for immunosuppression caused by AIDS comprising administering effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient who has AIDS.

129. A method of treating a patient for cancer comprising administering effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient followed by administration of a suitable effective amount of chemotherapeutic agent after about 4 hours.

130. The method of Claim 111 wherein the hematopoietic cells are mast cell progenitors.

131. The method of Claim 130 wherein the administeration is to modulate symptoms of allergy or asthma.

132. A method of increasing the level of red cells in the blood of a patient by administering forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient.

133. The method of Claim 124, 125, 126, 127, 128, 129, 130, 131 or 132 wherein the form of hyaluronic acid is sodium hyaluronate.

134. The method of Claim 133 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

135. The method of Claim 133 wherein the amount of the form of hyaluronic acid is at least about 6mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

136. The method of claim 133 wherein the method of treatment includes the administration of a plurality of dosages of the form of hyaluronan including at least one priming dosage in the amount of the form of hyaluronan less than about 3 mg/kg of patient body weight.

137. A method to mobilize any type of susceptible cell from one tissue to another, as a single agent or before/during other clinical procedures, as taught for hematopoietic and other types of normal or malignant cells, the method comprising administering an effective amount of a form of hyaluronan to a patient who will benefit therefrom wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

138. A method to mobilize hematopoietic cells before and during harvesting of tissue to be used for organ transplanations by the infusion of effective amounts of hyaluronan to a patient wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

139. A method of using ex-vivo hyaluronan perfusion to mobilize hematopoietic and dentritic-type cells out of an ex-vivo organ that has already been harvested from the donor by the infusion of an effective amount of hyaluronan to a patient wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

140. A method using hyaluronan infusion to treat host individuals about to receive an organ transplant prior to and during the transplantation procedure by the infusion of an effective amount of hyaluronan to a patient wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

141. A method using hyaluronan infusion to mobilize hematopoietic cells and dendritic-type cells away from/out of an organ graft that shows signs of immunologic rejection by the infusion of an effective amount of hyaluronan to a patient wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

142. A method to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells by infusing HA before and during the cytoreductive therapy administered prior to an autologous or allogeneic hematopoietic cell transplant in, for example, cancer patients such method comprises administration to a patient of an effective amount of hyaluronan to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells in patients benefiting from same wherein the form of hyaluronan is selected from hyaluronan and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

143. The method of Claim 137, 138, 139, 140, 141, 94 or 142 wherein the form of hyaluronic acid is sodium hyaluronate.

144. The method of Claim 143 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

145. A pharmaceutical composition for administration to an individual for the same purposes as recombinant GM-CSF or G-CSF is administered, comprising an

effective dosage amount of a form of hyaluronic acid having a molecular weight less than 750,000 daltons selected from the group consisting of hyaluronic acid and salts thereof and combinations thereof and pharmaceutically acceptable carrier and wherein said amount of the form of hyaluronic acid is between 1.5mg/kg to 12 mg/kg of patient body weight.

146. A pharmaceutical composition for administration to a human for the same purposes as recombinant erythropoietin, comprising an effective dosage amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons, and wherein said amount of the form of hyaluronic acid is between 1-5mg/kg to 12 mg/kg of patient body weight.

147. The composition of Claim 145 or 146 wherein the form of hyaluronic acid comprises at least about 1-5mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

148. The composition of Claim 145 or 146 wherein the form of hyaluronic acid is sodium hyaluronate comprising at least two dosages, a priming dosage amount and an additional dosage amount.

149. The composition of Claim 145 or 146 wherein the form of hyaluronic acid is at least about 12 mg/kg.

150. The composition of Claim 147 wherein the form of hyaluronic acid is sodium hyaluronate.

151. The composition of Claim 150 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

152. The composition of Claim 145 or 146 wherein the amount of the form of hyaluronan is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

153. The composition of Claim 145 or 146 wherein the dosage is a priming dosage in the amount of less than about 3mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

154. A pharmaceutical composition for administration to an individual to mobilize and stimulate the hematopoietic stem cells production and release comprising an effective amount of a form of hyaluronic acid selected from hyaluronic acid and salts thereof having a dosage of 1.5 mg/kg of patient body weight to 12 mg/kg of patient body weight.

155. The pharmaceutical composition of claim 154, wherein the hematopoietic stem cells comprises the cells selected from the group consisting of granulocytes, macrophages, CD34+ stem cells, monocytes, erythroblasts, polymorphonuclear cells, T-cells, B-cells and platelats.

156. The pharmaceutical composition of claim 155, wherein the form of Hyaluronic acid is sodium hyaluronate.

157. The pharmaceutical composition of claim 155, wherein the composition can be administrated to an individual orally, intravenously, or continuous infusion by placed a depot of the composition subcutaneously or intraperitonially.

158. The pharmaceutical composition of claim 157, wherein the molecular weight of the form of hyaluronic acid is between 200,000 daltons to 500,000 daltons.

159. The pharmaceutical composition of claim 157, wherein the molecular weight of the form of hyaluronic acid is between 50,000 daltons to 200,000 daltons.

160. The pharmaceutical composition of claim 158 or 159 further comprising a pharmaceutically acceptable carrier.

161. The pharmaceutical composition of claim 160, wherein the composition further comprises a therapeutic agent.

162. A method for the manufacture of a pharmaceutical composition for administration to an individual to mobilize cells from bone marrow or other tissue organs to blood, comprising mixing a mobilizing effective amount of a cell adhesion molecule with pharmaceutically acceptable salts thereof.

163. The method of claim 162, wherein the cells comprising hematopoietic stem cells, and dendritic-type cells.

164. The method of claim 163, wherein the hematopoietic stem cells comprises the cell selected from the group consisting of granulocytes, macrophages, CD34+ stem cells, monocytes, erythroblasts, polymorphonuclear cells, T-cells, B-cells and platelats.

165. The method of claim 164, wherein the mobilizing effective amount of a cell adhesion molecule is the effective fragment of hyaluronan, salts thereof and combinations thereof.

166. The method of claim 165, wherein the mobilizing effective amount of a cell adhesion molecule is the effective fragment of hyaluronan with a molecular weight ranging from 150,000 daltons to 750,000 daltons.

167. The method of claim 166, wherein the mobilizing effective amount of a cell adhesion molecule is the effective fragment of hyaluronan with a molecular weight ranging from 200,000 daltons to 500,000 daltons.

168. The method of claim 165, wherein the mobilizing effective amount of a cell adhesion molecule is the effective fragment of hyaluronan with a molecular weight ranging from 50,000 daltons to 200,000 daltons.

169. The method of claim 166, 167 or 168, wherein further mixing with a pharmaceutically acceptable carrier.

170. The method of claim 169, wherein further mixing with a therapeutic agent.

REMARKS

Claims 94 and 101 to 170 remain in the application. The fee of $4,560.00 USD for filing the application containing these claims is enclosed. This fee has been calculated in accordance with the Form PTO-1390 entitled "Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under 35 U.S.C. 371".

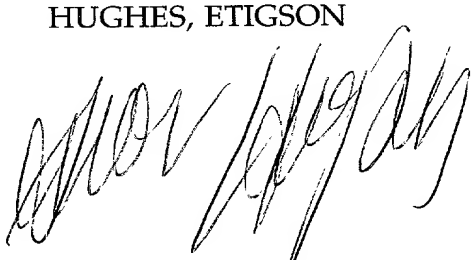
By virtue of the foregoing amendment, all the claims of PCT Application Serial No. PCT/CA97/00172 (the "172" application) have been cancelled except for Claim 94; and new Claims 101 to 170 have been added. The basis for the limitations of these new claims is present in the "172" application, as filed.

Applicants also enclose herewith an Information Disclosure Statement in accordance with 37 C.F.R. 1.97(b)(3), prior to issuance of a first Office Action on the merits. Applicant respectfully solicits the Examiner's consideration of the cited references and entry thereof into the record of this application.

If the Examiner wishes to discuss this matter with Applicant's agent, he/she is respectfully requested to contact Ivor M. Hughes or Samuel T. Tekie at (905) 771-6414 collect at her convenience.

Respectfully submitted,

HUGHES, ETIGSON



Ivor M. Hughes
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IMH/mse
Enclosures

TITLE OF INVENTION

Methods for cell mobilization using *in vivo* treatment with hyaluronan (HA).

FIELD OF THE INVENTION

The invention relates generally to methods using exogenous forms of hyaluronan (HA) for mobilizing hematopoietic cells to the circulation enabling various methods of treatment of humans, including mammals, including methods for obtaining hematopoietic cell transplantation, methods for treating immunosuppression, anemia, osteoporosis, methods for treating cancer, methods for treating allergy and asthma, methods for performing organ transplantation, methods for performing hematopoietic cell transplantation, methods for treating organ/tissue rejection, methods for treating autoimmunity and autoimmune-like conditions, and methods for in vitro fertilization, and in vivo fertility treatments.

BACKGROUND OF INVENTION

Hyaluronan (HA) is a ubiquitous glucosaminoglycan in the extracellular matrix, shown to play a central role in embyrogenesis, inflammation, wound healing, and tumour metastasis.

(Toole, B.P. (1990) *Hyaluronan and its binding proteins, the hyaladherins*. Curr. Opin. Cell. Biol. 2: 839-844.

Toole, B.P. (1982). *Development role of hyaluronate*. Conn. Tiss. Res 10: 93-100.)

Interaction between HA and RHAMM, a receptor for HA-mediated motility are required for motile behaviour of a wide variety of cells including sperm, fibroblasts, astrocytes, microglia and white blood cells.

(Entwistle, J. Zhang, S., Yang, B., Wong, C. Hall, C.L., Curpen, G., Mowat M., Greenberg, A.H., and Turley, E.A. (1995). *Cloning and characterization of the gene encoding the hyaluronan receptor RHAMM: the role of a secreted isoform in the regulation of focal adhesion formation*. Gene 163: 233-238

Yang, B., Yang, X. Zhang, S., Turley, M., Samuel, S.., Savani, R.C., Greenberg, A.H., and Turley, E.A. (1995). *Overexpression of the hyaluronan receptor RHAMM is transforming, and is required for H-ras transformation*. Cell 82: 19-28.

Masellis-Smith, A., Belch, A.R., Mant, M.J., Turley, E.A., and Pilarski., L.M. (1996). *Hyaluronan-dependent motility of B cells and*

leukemic plasma cells in multiple myeloma: Alternate usage of RHAMM and CD44. Blood 87: 1891-1899.

Turley, E.A., Belch, A.R., Poppema,, S., and Pilarski, L.M. (1993). *Expression and function of a receptor for hyaluronan-mediated motility (RHAMM) on normal and malignant B lymphocytes*. Blood 81: 446-453.

Pilarski, L.M. Miszta, H., and Turley, E.A. (1993). *Regulation expression of a receptor for hyaluronan-mediated motility RHAMM) on human thymocytes and T cells*. J. Immunol. 150: 4292-4302

S., K.B., McCoshen, J., Kredentser, J., and Turley, E. (1994). *The Regulation of Sperm Motility by a Novel Hyaluronan Receptor.* Fertility and Sterility 61: 935-940.

Turley, E.A., Sossain, M.Z., Sorokan, T., Jordan, L.M., and Nagy, J.I. (1994) *Astrocyte and microglial motility in vitro is functionally dependent on the hyaluronan receptor RHAMM*. Glia 12: 68-80)

The cells that populate the blood are all derived from multipotential (or pluripotential) stem cells present in bone marrow. Multipotential stem cells continually proliferate and renew themselves, but also give rise to common progenitor cells. Once committed, progenitor cells differentiate into immature precursor cells of the various blood cell lineages which, following further differentiation stages, eventually give rise to mature functional blood cells, such as erythrocytes, monocytes, lymphocytes, and polymorphonuclear cells. (Golub, E.S., Green, D.R. (1991) *Immunology A Synthesis*, 2:205; Kuby, J. (1997) *Immunology*, 3:50; Roitt, I., Brostoff, J., Male, D. (_____) *Immunology*, 4:2.1). Terminally differentiated blood cells generally lose their ability to proliferate - indeed mammalian erythrocytes and platelets contain no nuclei - and thus have finite lifetimes. Granulocytes may exist only for a matter of hours, whereas human erythrocytes remain in circulation for over 100 days. Although some lymphocytes have life-spans measured in years, most are short lived (for example, 3 days - 3 weeks). Therefore, to maintain steady-state numbers of particular blood cell types, there must be a continual production of these from the bone marrow. This process is known as haemopoiesis (haematopoiesis) or the haemopoietic process. While much remains to be learned, it is clear that many steps in the haemopoietic process (haemopoiesis) are controlled by certain cytokines (for example, GM-CSF and G-SCF and erythropoietin (EPO)), also known as haemopoietic growth factors, and by microenvironmental factors

including stromal cells and extra-cellular matrix components (for example, hyaluronan).

Clinically, the term "mobilization" usually refers to the process whereby cells leave the bone marrow and enter the blood. The mechanism whereby this occurs is not known by those skilled in the art. However, I believe mobilization can be viewed as the stimulation of de-adhesive behaviour by hematopoietic cells.

I believe that under normal circumstances, hematopoietic cells are anchored in their environment by receptors known as adhesion molecules. These adhesion molecules bind to components of the extracellular and cellular matrix within tissues to anchor the cell, or alternatively, to permit its migratory behaviour. Among the receptors thought to be important are those binding HA.

I believe mobilization involves two events: 1) a release from the anchoring interactions (de-adhesion) and 2) the stimulation of migratory behaviour. To reach the circulation from lymphoid tissue or the bone marrow a cell must "let go" of its anchoring interaction, activate adhesion receptors involved in migration (motile behaviour) and then actually locomote through tissue, penetrate endothelial cell linings and enter the blood vessel (intravasate). HA and receptors for HA are known to be involved in cell migration, motility and de-adhesion.

Most hematopoietic cells are anchored in the bone marrow or other lymphoid tissues. A stimulating/inducing event is required to mobilize them to the circulation as this is an active, not a passive process. Present practice involves administration of a variety of cytokines, often together with chemotherapeutic agents to mobilize hematopoietic cells to the blood. The mechanism for this is unknown. However, stem cell mobilization, the recruitment of hematopoietic stem cells into the blood where they can be easily harvested, is clinically performed using G-CSF and GM-CSF with or without chemotherapy.

(Weaver, C.H., Hazeltonn, B., Birch, R., Palmer, P., Allen, A., Schwwartzberg, L. and West, W. (1995). *An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy*. Blood 86: 3961-3969.

Boiron, J.-M., Marit, G., Faberes, C., Cony-Makhoul, P., Foures, C., Ferrer, A..-M., Cristol, G., Sarrat, A., Girault, D., and Reiffers, J. (1993) *Collection of peripheral blood stem cells in multiple myeloma following*

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Mobilized peripheral blood stem cell collections (PBSC) resulting from the use of G-CSF or GM-CSF are transplanted into, e.g. a cancer patient. These can be either the total population of the mobilized white blood cells or purified stem cells. Stem cells are those cells able to reconstitute the hematopoietic system of an organism, which requires self renewal of stem cells as well as differentiation to cells of the various hematopoietic lineages. The CD34 marker is characteristic of stem cells. Other cells that are mobilized include polymorphonuclear white blood cells (cells that mediate inflammation and clearance of pathogens), mononuclear white blood cells (lymphocytes and monocytes) and red blood cell progenitors (erythroblasts).

Mobilization of CD34+ stem cells is a rapidly expanding clinical technique for obtaining material for autologous or allogeneic hematopoietic transplantation. Mobilization of polymorphs is a valuable adjunct to heavy chemotherapy to maintain innate defense mechanisms. Currently, both methods rely on mobilization by growth factors (G-CSF or GM-CSF) which is expensive, causes bone pain, and has unknown side effects for normal donors. It takes up to about 4 weeks of treatments to collect sufficient material for a transplant. After growth factor treatment, CD34+ cells reach a maximum level of 2-4% in blood.

The mature cells of the haemopoietic system include erythrocytes, polymorphonuclear-cells (PMN), lymphocytes, monocytes, macrophages, osteoblasts, osteoclasts, mast cells, and platelets. These all have a limited life-span, and must be replaced as they die. To achieve a balance between cell death and renewal, the bone marrow must not only continuously provide progenitor cells, but also control the commitment of these to the various lineages so that the correct proportions of mature cells are produced. The basic control mechanisms, especially of the earliest stages of haemopoiesis, are as yet poorly understood. There appears to be some compartmentalization of the marrow, and microscopic 'nests' of

particular precursor cells have been identified. However, it has been shown that the survival and proliferation of stem and progenitor cells is dependent upon the presence of accessory cells which *in vitro* form into an adherent 'stromal' layer. In the absence of the stromal layer, stem and progenitor cells die and so it appears the stromal cells support proliferation and differentiation by intercellular interactions including production of growth factors into the extracellular milieu. In culture, stromal cells have been shown to produce GM-CSF, M-CSF, and a megakaryocyte-colony stimulating factor (or molecules functionally equivalent to these). It is widely believed that such growth factors (cytokines) are involved in haemopoiesis, but their exact role(s) in self-renewal of stem cells, differentiation of stem cells into common progenitor cells, and the proliferation and differentiation of committed progenitor cells, remains unclear. More definite roles of these cytokines in the growth stimulation and development of later-stage precursors have been evinced by the use of *in vitro* colony-forming culture systems introduced by Metcalf and colleagues in the 1970s. In these experimental systems multipotential stem cells, progenitors, or precursors are suspended in the absence of stromal cells in semi-solid agar growth medium. Without the addition of exogenous cytokines, the cells die. However, they can be stimulated to grow, multiply, and differentiate to form colonies of various blood cell lineages by adding into the growth medium dilutions of certain supernatants obtained from activated leukocytes or by addition of the now readily available purified recombinant cytokines including GM-CSF. Furthermore, injection of recombinant cytokines into experimental animals, and into patients in clinical trials to assess therapeutic potential of individual cytokine products, has shown that IL-3, GM-CSF, and G-CSF stimulate the production of white cells such as granulocytes and monocytes, thus lending support for physiological roles of such cytokines. In addition, it has also become apparent that these cytokines not only support the growth and differentiation of immature blood cells, but also in many instances are effector molecules for the functional activation of mature cells.

The molecular cloning of both murine and human homologues of IL-3, GM-CSF, G-CSF, M-CSF, IL-5, and EPO has been accomplished.

Of the four 'granulocyte-macrophage' CSFs, GM-CSF was the first to be isolated and characterized. GM-CSF was shown to induce the

proliferation of murine bone marrow - or spleen-derived haemopoietic cells containing granulocyte and macrophage progenitors giving rise to colonies containing mainly granulocyte and macrophage precursors. In this respect, GM-CSF appears to share biological properties with the subsequently characterized IL-3. However, more recent studies suggest that GM-CSF acts on 'later-stage' multipotential cells than IL-3. Also, GM-CSF appears to be less active than IL-3 in stimulating the proliferation of erythroid and megakaryocytic precursors. Nevertheless, like IL-3, GM-CSF can be shown to have activities in mature cells of the granulocyte and macrophage lineages.

GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor) acts directly and selectively on granulocyte/macrophage progenitors to stimulate growth and differentiation *in vitro* of cells belonging to these lineages, e.g. neutrophils, eosinophils, macrophages. These pleiotropic activities have also been demonstrated for recombinant GM-CSF. Besides regulation of the proliferation and differentiation of the progenitor/precursor cells of the myeloid lineage, GM-CSF has also been shown to activate the functions of mature myeloid cell types. For example, GM-CSF has been found to induce macrophage tumoricidal activity against the malignant melanoma cell line, A375. IFNγ can also behave as a macrophage activating factor, but in contrast to GM-CSF requires an additional secondary stimulus, e.g. bacterial LPS, to evoke tumoricidal activity. In addition, GM-CSF activates macrophages to inhibit the replication of *Trypanosoma cruzi* (a unicellular parasite that is the aetiological agent of Chagas disease, or American trypanosomiasis) and increases respiratory oxidative processes. Furthermore, the replication of HIV-1 in the human monocytic cell line U937 has been shown to be moderately inhibited by GM-CSF, and more effectively by the combination of GM-CSF and IFNγ. These results suggest that GM-CSF could have a potential physiological role in eosinophils and macrophage activation and thus possibly could be used prophylactically or therapeutically against a range of microbial agents that replicate in macrophages.

In neutrophils and eosinophils, GM-CSF stimulates a number of functions. In particular, GM-CSF enhances phagocytosis of bacteria and yeasts by neutrophils. Purified recombinant human GM-CSF has also been shown to enhance the cytotoxic activity of neutrophils and eosinophils against antibody-coated target cells. These observations and

others in which the anti-microbial functions of neutrophils and eosinophils are increased by GM-CSF, strongly suggest an important role for this mediator in host defence.

When mice are repeatedly injected intraperitoneally with recombinant murine GM-CSF, there is a rapid and sustained increase in the number and functional activity of peritoneal macrophages, granulocytes (neutrophils and eosinophils) as well as increased numbers of circulating monocytes. (GM-CSF usually takes about two weeks to act.) Marked increases in neutrophil, eosinophil, and monocyte numbers have also been observed following injection of recombinant human GM-CSF into AIDS patients and non-human primates. However, there may be complications associated with GM-CSF therapy. Metcalf and colleagues have shown that transgenic mice containing a constitutively expressed murine GM-CSF gene have pathological lesions soon after birth in various tissues, including lens, retina, and striated muscle, resulting from activated-macrophage infiltration. Thus, chronic macrophage activation in GM-CSF therapeutic schedules should be avoided. (Activated macrophages are known to produce a number of inflammatory mediators including cytokines such as TNFα and IL-1 which may induce tissue damage.)

In contrast to its growth-stimulating effects, GM-CSF can act as a differentiation factor. Its actions on mature macrophages and neutrophils, for example, might be considered as consequences of its differentiation-inducing capacity. One way to limit the proliferation of tumour cells is to decouple growth-factor-driven self-renewal from growth-factor-induced differentiation. In other words, the more 'differentiated' tumour cells become, the less able they are to multiply. In this regard, GM-CSF has been shown to induce differentiation of the myeloid leukaemic cell line HL60 and suppress its self-renewal. However, in several other studies, GM-CSF stimulated the proliferation of HL60 cells. Differentiation can be monitored by measuring expression of various plasma membrane-associated antigens, e.g. CD14 (monocyte/macrophage marker) and CD57 (NK cell marker). These have been reported to be induced by GM-CSF in small cell lung cancer (SCLC) cell lines, suggesting that SCLC has a myeloid cell origin. This would be consistent with a proposal that SCLC arises from macrophage precursors which infiltrate damaged lung tissues, such as occur in heavy smokers. The ready availability of recombinant human GM-CSF and the limited

distribution of GM-CSF receptors to cells of the myeloid and possibly erythroid lineages may thus help to define the histological origin of tumours, and suggests alternative therapeutic modalities for the treatment of cancers such as SCLC.

It thus appears that while the use of Granulocyte-macrophage colony stimulating factor (GM-CSF) has been used as a stimulant for the production of stem cells, progenitor cells, precursor cells, accessory cells and macrophages there are a substantial number of disadvantages in its use, those discussed above and the appearance of bone pain, fever, myalgia and erythema in patients to whom cytokines such as GM-CSF and G-CSF were administered, which make the use of GM-CSF and G-CSF not as desirable.

It is therefore an object of this invention to provide the use of another and other compounds which provide similar effects as GM-CSF and G-CSF but with lesser side effects.

It is a further object of this invention to provide such compounds in suitable dosages for effective and safe use.

It is still a further object of this invention to provide improved treatments and regimens of treatment.

It is a further object of the invention to provide a novel use for hyaluronan (HA) for mobilizing cells such as hematopoietic cells.

Further and other objects of the invention will be realized by those skilled in the art from the following summary of invention and detailed description of embodiments thereof.

SUMMARY OF THE INVENTION

The invention provides for a novel use for forms of hyaluronan (HA) for mobilizing hematopoietic cells from bone marrow and other tissues into the circulation.

The invention also provides for the novel use for forms of HA for mobilizing dendritic-type cells from bone marrow and other tissues into the circulation.

The invention also provides for novel use for forms of HA for activating/stimulating stromal cells to facilitate mobilization.

The invention also provides for novel use for forms of HA for releasing cancer cells into the blood.

The forms of HA include hyaluronan and pharmaceutically acceptable salts thereof.

The term "hematopoietic cells" is meant to include all types of hematopoietic cells throughout their differentiation from self-renewing hematopoietic stem cells through immature precursor cells of the various blood lineages to and including the mature functioning blood cells as would be understood by persons skilled in the art.

The term "dendritic-type cells" is meant to include cells in the circulation, the bone marrow and other tissues, including those cell types involved in antigen presentation.

The term "stromal cells" is meant to include the accessory cells that make up the microenvironment of hematopoietic cells, including endothelial cells, adipocytes, fibroblasts, reticular cells, and epithelial cells, and all functionally-like cells.

The terms "individual" or "patient" are meant to encompass all species of mammals. Although examples below may refer to humans, a person skilled in the art would know this is also applicable to other species of mammals.

The term "stimulate" is meant to be equivalent to the term "activate".

The invention further provides for methods for mobilizing hematopoietic cells comprising administering forms of HA *in vivo*.

The invention further provides a method for generating hematopoietic cells (for example, stem cells) for transplantation comprising administering forms of HA *in vivo*, and harvesting the cells to be transplanted from the peripheral blood.

The invention further provides a method for mobilizing dendritic-type cells.

The invention further provides a method for activating/stimulating stromal cells.

The invention further provides methods for treating immunosuppression caused by chemotherapy comprising administering forms of HA to individuals who have undergone chemotherapy.

The invention further provides methods for treating immunosuppression and/or immunodeficiency, for example, associated with AIDS, comprising administering forms of HA to individuals who are immunosuppressed or immunodeficient.

The invention further provides methods for treating osteoporosis comprising administering forms of HA to individuals who suffer from osteoporosis.

The invention further provides methods for treating asthma and allergy comprising administering forms of HA to individuals who suffer from asthma and allergy.

The invention further provides methods for treating acquired anemia comprising administering forms of HA to individuals who suffer from acquired anemia (such as blood loss, iron deficient anemia, anemia accruing post-surgery, infection-related anemia, insulin related anemia, low hemoglobin anemias of pregnancy or from red blood cell production). Thus, this invention provides for the administration of forms of HA for the same use as erythropoietin is administered.

The invention further provides methods to release cancer cells from the bone marrow and other tissues into the blood.

According to an aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the same use as recombinant G-CSF and/or GM-CSF including the production/release of stem, progenitor and other hematopoietic cells.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of hematopoietic cell production/release, e.g. stem cells.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of dendritic-type cell production/release and the stimulation of other tissue based antigen-presenting cells production/release.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation/activation of stromal cells.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for sodium hyaluronate) enhance the release of cancer cells from the bone marrow and other tissue into the blood.

In support of my conclusions as to what constitutes my invention, I have conducted tests for which the results are set out as examples herein. Additionally, I have now re-examined tests previously conducted and ongoing tests and determined that my unobvious results, conclusions and

thus my invention are substantiated by these additional materials. HA receptors are known to be expressed on nearly all types of hematopoietic cells. Tests have shown that HA receptors able to bind HA are expressed by T lymphocytes, B lymphocytes and monocytes from normal individuals or those with an inflammatory disease (restenosis or inflammatory bowel disease); the receptors involved include RHAMM and CD44. Malignant B cells also express these receptors and utilize them for binding HA as well as in motile behaviour (Masellis Smith et al, BLOOD 1996). Testing has also shown that human thymocytes (immature T cells) have few HA receptors but that interaction with HA causes redistribution of HA receptors (mainly RHAMM) to the cell surface where it is now able to bind HA and to interact with HA to promote thymocyte motility. Testing has also shown that thymocytes have a pool of cryptic HA receptors that are able to bind HA when exposed experimentally, and which can be redistributed to the surface for functional use.

Thus, I have determined that HA is able to upregulate HA receptors, in particular RHAMM, and that these newly expressed receptors actually mediate cell motility. This is an in vitro model of cell mobilization, in this case modeling events that cause thymocytes to leave the solid organ, the thymus, for the blood, analogous to the predicted behavior of HA infused in vivo, as claimed in this invention to cause hematopoietic cells of many types to leave the bone marrow or other tissue and enter the blood. This testing therefore also supports my invention which deals with events in vivo. I believe the infused HA causes redistribution of HA receptors on hematopoietic cells of many types (stem cells and cells at all differentiation stages within hematopoietic lineages) thus increasing their surface density. These receptors then interact with HA to cause de-adhesion and initiation of motile behavior required for migration to the blood (which, I believe, is required for hematopoietic cell mobilization as understood in the clinical terminology). While I believe that this event takes place as described, my invention can be used irrespective of the actual mechanism of mobilization.

Additionally, $CD34^+$ stem cells express HA receptors and can bind HA, providing further in vitro support for their mobilization from the bone marrow to the blood by HA infusion.

Peripheral blood T cells have cryptic HA receptors, are able to weakly bind HA, and undergo motile behavior that is inhibited by

antibody to the HA receptor RHAMM even though this receptor is not expressed at an otherwise detectable density of the T cell surface. The fact that RHAMM mediates the motility indicates that HA has upregulated cryptic RHAMM on mature blood T cells as has been demonstrated for immature T cells. It also shows that HA will, I believe, mobilize T cells from lymphoid organs and tissue other than the bone marrow (including the spleen, lymph nodes, Peyer's patches, gut–associated lymphoid tissue and skin associated lymphoid tissue).

Osteoclasts, the cells that dissolve bone and which participate in pathological destruction of bone mass, express the HA receptor CD44, while osteoblasts and osteoprogenitors, the cells that produce bone mass, have only a low amount of CD44. Therefore, I believe, this indicates that osteoclasts and thus bone destruction are preferentially affected by HA infusion as compared to osteoblasts and osteoprogenitors. Osteoporosis and other conditions characterized by reduced bone mass and resultant increased bone fragility, will thus be modulated by HA infusion. Such modulation will preferentially impact on cells responsible for bone destruction while sparing those responsible for bone production, supporting the use of HA infusion in bone diseases such as osteoporosis.

Further, malignant lymphocytes of the B lineage (lymphoma, multiple myeloma, hairy cell leukemia) express RHAMM and utilize RHAMM to undergo motile behavior. In this case motility is a behavior required for cancer cell spread as well as for migration of cancer cells to and from the bone marrow and other lymphoid organs. Thus, the form of HA, I believe, will mobilize cancer cells, thus facilitating their targeting by therapy.

In many cancers, bone localized cancer cells, or bone metastases are a serious complication of the cancer. Although chemotherapy does reach the bone spaces, it seems inevitable that some regions of the bone are less vascularized than others and that some pockets of malignant disease escape chemotherapeutic agents. A number of studies indicate that tumor cells aggregated into a tumor mass are more drug resistant than those in single cell suspensions. Mobilization of tumor cells into the blood will release them from any tumor aggregates in the marrow or other sites and, I believe, will render them drug sensitive.

Multiple myeloma is a good example of a cancer with large numbers of bone localized malignant cells, while lymphoma and breast cancer include bone metastases which depend on migration from the

primary tumor mass through the blood to the bone marrow. The bone marrow is served only by the blood so any traffic to or from the marrow must occur via the blood. In breast cancer, multiple myeloma, and some lymphomas, the HA receptor RHAMM is expressed and identifies circulating cancer cells. In myeloma, the bone marrow localized cancer cells express RHAMM and CD44. For those cancers originating as a solid mass that is usually surgically removed, infusion of HA at the time of surgery, I believe, will prevent surgically dislocated cancer cells from migration through the blood, for example, into the bone or any other tissue site, thus reducing the risk of iatrogenic spread. These cancer cells are now more vulnerable, I believe, to chemotherapy. For those patients with bone tumor cells, I believe, infusion with HA will cause their mobilization into the periphery where they will be more readily attacked, will be exposed to potentially higher doses of therapeutic agents, and where they will now be susceptible to agents that cannot easily enter the bone marrow.

One such possible treatment is the use of combinations of hyaluronan and liposomes and/or any suitable therapeutic agent which, for example, may be bound to hyaluronan. Hyaluronan may be equally used as a targeting and delivery moiety for any suitable agent. See PCT Application WO 91/04058. Treatment may also be by administration of chemotherapeutic agents or other types of therapy.

According to another aspect of the invention, a method is provided whereby a form of HA is infused to a patient to mobilize cancer cells from bone marrow or solid tissues into the blood where the cancer cells exist as single cell suspensions and are rendered more drug sensitive and/or are more effectively attacked by a therapeutic agent and/or removed by some physical procedure such as leukapheresis.

Suitable amounts of the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof may be in the order of between about 1.5mg/kg of body weight and about 12mg/kg of body weight, for example, about 6mg/kg of patient body weight to whom the form of hyaluronic acid is administered (for example, by intravenous infusion or other suitable manner) or a greater amount, such as about 8mg/kg of patient body weight and about 12mg/kg of patient body weight, to whom the form of hyaluronic acid is administered. Thus, suitable dosage amounts for a 70kg person, comprise at least about 105mg, for

example, about 420mg of the form of hyaluronic acid and for example, 840mg of the form of hyaluronic acid.

Depending on the cells to be mobilized, different treatment regimens of the dosages may be administered. The following regimens of treatment may be reviewed as follows:

(a) a single dose selected from the above range may, for example, selectively mobilize the desired cell type;

(b) sequential infusions of the same dosage amount of the form of HA (for example, 6mg/kg given weekly for a month);

(c) sequential infusions with differing amounts in any order. Where patients are given a regimen of treatment over a period of time for example, smaller (lesser) amounts/kg of patient body weight over a period of time (for example, every few days or once a week for a number of weeks), lesser amounts than 6 mg/kg may be used to achieve the same effect. The patient may even be "primed" to start the treatment by giving smaller/lesser dosages which, by themselves, may not be effective for the cell type (but is effective for other cell types). Such priming amounts may for example, be 1.5 mg/kg or 3.0 mg/kg of body weight.

The form of hyaluronic acid may be administered in any suitable carrier such as sterile water or saline. The stimulatory effect usually commences as early as one hour after administration of a form of hyaluronic acid and continues for at least about 72 hours and in cases the effects were still visible after 7 days.

One form of hyaluronic acid and/or pharmaceutically acceptable salts thereof (for example sodium salt) suitable for use with Applicant's invention is an amount having the following specifications/characteristics:

TESTS	SPECIFICATIONS	RESULTS
pH	5.0 to 7.0 at 25 degrees C.	6.0
Specific Gravity	0.990 to 1.010 at 25 degrees C.	1.004
Intrinsic Viscosity	4.5 to 11.0 dL/g.	7.07
Molecular Weight	178,000 to 562,000 daltons	319,378 daltons
Sodium Hyaluronate	9.0 to 11.0 mg/mL. Positive	9.9 mg/ML
Assay and Identification		Positive

Another such amount may comprise:

TESTS	SPECIFICATIONS
1. Description	White or cream odourless powder
2. Identification (IR Spectrum)	Conforms to Ref. Std. Spectrum
3. pH (1% solution)	5.0 to 7.0
4. Loss on Drying	NMT 10%
5. Residue on Ignition	15.0% to 19.0%
6. Protein Content	NMT 0.1%
7. Heavy Metals	NMT 20 ppm
8. Arsenic	NMT 2 ppm
9. Residual Solvents	
a) Formaldehyde	NMT 100 ppm
b) Acetone	NMT 0.1%
c) Ethanol	NMT 2.0%
10. Sodium Hyaluronate Assay (dried basis)	97.0 to 102.0%
11. Intrinsic Viscosity	10.0 to 14.5 dL/g
12. Molecular Weight	500,000 to 800,000 daltons
13. Total Aerobic Microbial Count (USP 23)	NMT 50 microorganisms/g
14. Escherichia coli (USP 23)	Absent
15. Yeasts and Moulds (USP 23)	NMT 50 microorganisms/g
16. Bacterial Endotoxins (LAL) (USP 23)	NMT 0.07 EU/mg

Another such amount is available from Hyal Pharmaceuticals Limited and comes in a 15 ml vial of Sodium hyaluronate 20mg/ml (300mg/vial - Lot 2F3). The sodium hyaluronate amount is a 2% solution with a mean average molecular weight of about 225,000. The amount also contains water q.s. which is triple distilled and sterile in accordance with the U.S.P. for injection formulations. The vials of hyaluronic acid and/or salts thereof may be carried in a Type 1 borosilicate glass vial closed by a butyl stopper which does not react with the contents of the vial.

The amount of hyaluronic acid and/or salts thereof (for example sodium salt) may also comprise the following characteristics:

a purified, substantially pyrogen-free amount of hyaluronic acid obtained from a natural source having at least one characteristic selected from the group (and preferably all characteristics) consisting of the following:

i) a molecular weight within the range of 150,000-225,000;

ii) less than about 1.25% sulphated mucopoly-saccharides on a total weight basis;

iii) less than about 0.6% protein on a total weight basis;

iv) less than about 150 ppm iron on a total weight basis;

v) less than about 15 ppm lead on a total weight basis;

vi) less than 0.0025% glucosamine;

vii) less than 0.025% glucuronic acid;

viii) less than 0.025% N-acetylglucosamine;

ix) less than 0.0025% amino acids;

x) a UV extinction coefficient at 257 nm of less than about 0.275;

xi) a UV extinction coefficient at 280 nm of less than about 0.25; and

xii) a pH within the range of 7.3-7.9. Preferably, the hyaluronic acid is mixed with sterile water and the amount of hyaluronic acid has a mean average molecular weight within the range of 150,000-225,000 daltons. More preferably, the amount of hyaluronic acid comprises at least one characteristic selected from the group (and preferably all characteristics) consisting of the following characteristics:

i) less than about 1% sulphated mucopolysaccharides on a total weight basis;

ii) less than about 0.4% protein on a total weight basis;

iii) less than about 100 ppm iron on a total weight basis;

iv) less than about 10 ppm lead on a total weight basis;

v) less than 0.00166% glucosamine;

vi) less than 0.0166% glucuronic acid;

vii) less than 0.0166% N-acetylglucosamine;

viii) less than 0.00166% amino acids;

x) a UV extinction coefficient at 257 nm of less than about 0.23;

xi) a UV extinction coefficient at 280 nm of less than 0.19; and

xii) a pH within the range of 7.5-7.7

Applicants may also use sodium hyaluronate produced and supplied by LifeCore™ Biomedical, Inc., having the following specifications:

Characteristics	Specification
Appearance	White to cream colored particles
Odor	No perceptible odor
Viscosity Average Molecular Weight	< 750,000 Daltons
UV/Vis Scan, 190-820nm	Matches reference scan
OD, 260nm	< 0.25 OD units
Hyaluronidase Sensitivity	Positive response
IR Scan	Matches reference
pH, 10mg/g solution	6.2 - 7.8
Water	8% maximum
Protein	< 0.3 mcg/mg NaHy
Acetate	< 10.0 mcg/mg NaHy

Heavy Metals, maximum ppm

As	Cd	Cr	Co	Cu	Fe	Pb	Hg	Ni
2.0	5.0	5.0	10.0	10.0	25.0	10.0	10.0	5.0

Microbial Bioburden	None observed
Endotoxin	< 0.07EU/mg NaHy
Biological Safety Testing	Passes Rabbit Ocular Toxicity Test

Another amount of sodium hyaluronate proposed to be used is sold under the name Hyaluronan HA-M5070 by Skymart Enterprises, Inc. having the following specifications:

Specifications' Test Results

Lot No.	HG1004
pH	6.12
Condroitin Sulfate	not detected
Protein	0.05%
Heavy Metals	Not more than 20 ppm
Arsenic	Not more than 2 ppm
Loss on Drying	2.07%
Residue on Ignition	16.69%

Intrinsic Viscosity	12.75 dl/s (XW: 679,000)
Nitrogen	3.14%
Assay	104.1%
Microbiological Counts	80/g
E. coli	Negative
Mold and Yeast	Not more than 50/g

Other forms of hyaluronic acid and/or its salts may be chosen from other suppliers and those described in prior art documents provided they are suitable.

The following references teach hyaluronic acid, sources thereof, and processes for the manufacture and recovery thereof which may be suitable.

United States Patent 4,141,973 teaches hyaluronic acid fractions (including sodium salts) having:

"(a) an average molecular weight greater than about 750,000, preferably greater than about 1,200,000 - that is, a limiting viscosity number greater than about 1400 cm^3/g., and preferably greater than about 2000 cm^3/g.;

(b) a protein content of less than 0.5% by weight;

(c) ultraviolet light absorbance of a 1% solution of sodium hyaluronate of less than 3.0 at 257 nanometers wavelength and less than 2.0 at 280 nanometers wavelength;

(d) a kinematic viscosity of a 1% solution of sodium hyaluronate in physiological buffer greater than about 1000 centistokes, preferably greater than 10,000 centistokes;

(e) a molar optical rotation of a 0.1 - 0.2% sodium hyaluronate solution in physiological buffer of less than -11×10^3 degree - cm^2/mole (of disaccharide) measured at 220 nanometers;

(f) no significant cellular infiltration of the vitreous and anterior chamber, no flare in the aqueous humour, no haze or flare in the vitreous, and no pathological changes to the cornea, lens, iris, retina, and choroid of the owl monkey eye when one milliliter of a 1% solution of sodium hyaluronate dissolved in physiological buffer

is implanted in the vitreous replacing approximately one-half the existing liquid vitreous, said HUA being

(g) sterile and pyrogen free and

(h) non-antigenic."

Canadian Letters Patent 1,205,031 (which refers to United States Patent 4,141,973 as prior art) refers to hyaluronic acid fractions having average molecular weights of from 50,000 to 100,000; 250,000 to 350,000; and 500,000 to 730,000 and discusses processes of their manufacture.

Where high molecular weight hyaluronic acid (or salts) is used, it should be treated to permit administration and ensure no coagulation or blockage.

As there is no toxicity of the form of hyaluronic acid, the form of hyaluronic acid may be administered in doses in excess of 12mg/kg of body weight, for example, in excess of 1000mg/70kg person and even up to amounts of 3000mg/70 kg person without adverse toxic effects.

Thus, according to another aspect of the invention, a method of treatment is provided comprising the administration to a mammal (human) of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of hematopoietic cells as measured by phenotypic, physical or chemical properties or any other characteristics used by those skilled in the art to identify a given cell type.

Thus, according to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid is provided for enhancing (stimulating) the production/release of hematopoietic cells as measured by phenotypic, physical or chemical properties or any other characteristics used by those skilled in the art to identify a given cell type.

Thus, according to another aspect of the invention, a method of treatment is provided comprising the administration to a mammal (human) of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of dendritic and related antigen presenting cells (dendritic-type cells) as measured by phenotypic, physical or chemical properties or any other characteristics used by those skilled in the art to identify a given cell type.

Thus, according to another aspect of the invention, a method of treatment is provided comprising the administration to a mammal (human) of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), to activate/stimulate stromal cells in the bone marrow and other tissues.

Thus, according to another aspect of the invention, a method of treatment is provided comprising the administration to a mammal (human) of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), to release cancer cells from the bone marrow and other tissues to the blood.

Thus, according to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid is provided for enhancing (stimulating) the production/release of dendritic and related antigen presenting cells as measured by phenotypic, physical or chemical properties or any other characteristics used by those skilled in the art to identify a given cell type.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a mammal (e.g. human) for enhancing (stimulating) the production/release of hematopoietic cells.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a mammal (e.g. human) in order to activate/stimulate stromal cells in the bone marrow and other tissues.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a mammal (e.g. human) in order for the release of cancer cells from the bone marrow and other tissues into the blood.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a mammal (e.g. human) for enhancing (stimulating) the production/release of dendritic and related antigen presenting cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of hematopoietic cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of dendritic and related antigen presenting cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided to activate/stimulate stromal cells in the bone marrow and other tissues.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided to release cancer cells from the bone marrow and other tissues into the blood.

Thus, by administering effective amounts of the forms of hyaluronic acid, patients can be treated with the form of hyaluronic acid which is safe and non-toxic and the patient does not suffer the adverse side effects of recombinant GM-CSF or G-CSF treatment, yet achieves results that are obtained by the administration of recombinant GM-CSF or G-CSF. By administering a regimen of treatment comprising a single dose or a plurality of dosages of hyaluronic acid over a period of time (for example, several weeks) or dosages comprising an amount or amounts which is/are lesser amount(s) followed by amounts which are greater amounts, the patient can be treated. Lesser amounts than the amounts used without priming may be effective in the patient to stimulate the production/release of the cells, the activation, or the release, when the patient is primed. For example, suitable dosage amounts may be 6mg/kg of patient body weight or 12 mg of the form of hyaluronic acid/kg patient body weight. A suitable regimen may also comprise a suitable amount (for example 1.5 mg of the form of hyaluronic acid/kg patient body weight or 3.0 mg/kg for "priming" purposes also followed by administration of

another effective amount (for example, about 6 mg/kg, 10 mg/kg or more after a pre-determined interval or intervals. Another suitable regimen of sustained treatment may be provided as follows:

Week 1: 1.5 mg/kg;
Week 2: (7 days later) - 3.0 mg/kg;
Week 3: (7 days later) - 6 mg/kg;
Week 4: (7 days later) - 12 mg/kg;

The treatment at Week 3 or 4 may be continued in Weeks 5, 6, 7, etc. for as long as required. Any of the treatments may be continued for as long as required.

Thus, the invention provides a novel use for HA as an agent to mobilize hematopoietic and dendritic-type cells. The invention further provides for methods of mobilizing hematopoietic and dendritic-type cells, comprising treatment of subjects with HA. The invention can be used in a variety of applications for which it is necessary or desirable to mobilize hematopoietic and dendritic-type cells, including, but not limited to: obtaining material for transplantation; post-chemotherapy mobilization of granulocytes and monocytes; mobilization of CD4+ T cells from solid lymphoid organs into the blood of AIDS patients.

In an embodiment, mobilization of hematopoietic cells, including polymophonuclear cells, erythroblasts, plasma cells, early stage monocytes, T cells, B cells and stem cells, I believe, was achieved by the sequential intravenous infusion of increasing doses of HA (having a molecular weight of 200,000 to 300,000 daltons determined according to the protein-standard isolated from for example, *Streptomyces*), for over a period of 4 weeks as outlined herein.

This embodiment can be varied and still give equivalent results, and the embodiment can be further optimized for various applications, as indicated herein.

It is anticipated that the administration of HA as smaller or larger fragments could also be used to practise the invention, and their use is included in the invention. HA can be isolated in average MW forms such as the 200,000 to 300,000 MW average amounts used herein, or even smaller molecular weights.

The possibility exists that smaller fragments of HA, less than 200,000 to 300,000, could be more potent in mobilizing hematopoietic and dendritic-type cells for the following reasons. HA breaks down quickly in the circulation and is rapidly cleared by liver endothelial cells. I believe

that the HA used, 200,000 to 300,000 MW, is rapidly degraded into smaller fragments that may have increased biological activity in stimulating migratory behaviour, as well as more effective in mediating de-adhesion (release of anchoring). I believe that smaller HA fragments can more easily enter the bone marrow than high MW fragments, which might not be able to traverse through the bone marrow sinus areas where exchange between blood and bone marrow compartments must occur. However, since HA is broken down in vivo, the use of larger fragments might be equally effective. The optimum size of HA for infusion can be determined by selecting HA in various MW ranges (e.g. 25,000-50,000, 50,000-100,000 M.W., etc.).

Doses of HA include, but are not limited, to the range of about 1.5 mg/kg to about 12 mg/kg separately, and together in sequential combinations (i.e. multiple infusions of only one concentration, and multiple infusions each at different concentrations).

For example, because 12 mg/kg provided a major effect (see herein), it would be apparent to persons skilled in the art to try increasing doses of HA to find optimal doses for each use. This can be done simply by administering increasing doses of HA to subjects, and analyzing blood samples taken for example, as described herein. Furthermore, rather than sequentially increasing the dose on a weekly basis, HA could be infused weekly (or at other intervals) at an optimal concentration which might be higher or lower than 12mg/kg depending on the cells to be mobilized. Different frequencies and durations of HA administration can also be examined.

Different routes of administration in addition to intravenous infusion are effective. For example, a "depot" of HA placed subcutaneously or intraperitoneally would provide continuous infusion over a prolonged period and would be a convenient and effective method of providing HA. If HA is administered subcutaneously or intraperitoneally, for example continuous infusion could be achieved and monitoring the subject over time. Alternatively, HA could be administered orally. The invention includes administration of HA by such means and all others as would be understood by persons skilled in the art.

Different protocols of HA administration including variations in size of HA, dosage, route and duration of HA administration will mobilize different populations of hematopoietic and dendritic-type cells.

For example, a protocol which optimally mobilizes CD34+ stem cells may be somewhat different from one which is optimal in mobilizing T cells or tumour cells. Thus, the protocol can be optimized for a particular desired application, by administering HA under different conditions, and then monitoring the output of the desired subset of hematopoietic cells in the blood as indicated herein. The time after infusion should be monitored optimal recovery or induction of specific cell populations in the blood, because, as noted in the example herein, the appearance of different cell types occurred sequentially over a period of about a week after infusion. The pattern indicated early (4 hr) release of polymorphonuclear cells and erythroblasts (relatively late stage red cell progenitors which are nucleated), later release of stem cells, small lymphocytes, and plasma cells (24-72 hours) and still later release of monocytoid cells (7 days).

The subjects herein were human subjects. HA would also be effective in primates or other mammals for the mobilization of hematopoietic cells to be used in xenotransplantation or for collecting hematopoietic cells from genetically altered animals (e.g. a pig genetically engineered to express human major histocompatability antigens on their hematopoietic cells).

The use of HA as an agent to mobilize hematopoietic cells is illustrated in the following:

1. HA infusion can be used to generate a source of hematopoietic stem cells for allogeneic or autologous transplantation. Such transplantation is frequently used to restore the hematopoietic system of cancer patients after myeloablative chemotherapy and radiotherapy. Stem cell donors (either the cancer patient prior to chemotherapy or an allogenic donor) can be treated with HA using a generally effective protocol such as the one described herein, or a protocol which has been specifically optimized to yield the maximum number of CD34+ stem cells. PBMC collections can be treated, frozen, and infused into patients using clinical protocols which are well known in the art (for detailed examples of such protocols, see References below:).

   (Weaver, C.H., Hazeltonn, B., Birch, R., Palmer, P., Allen, A., Schwwartzberg, L. and West, W. (1995). *An analysis of engraftment kinetics as a function of the CD34 content of*

peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood 86: 3961-3969.

Boiron, J.-M., Marit, G., Faberes, C., Cony-Makhoul, P., Foures, C., Ferrer, A..-M., Cristol, G., Sarrat, A., Girault, D., and Reiffers, J. (1993) *Collection of peripheral blood stem cells in multiple myeloma following single high-dose cyclophosphamide with and without recombinant human granulocyte-macrophage colony-stimulating factor (rh GM-CSF)*. Bone Marrow Transplantation 12: 49-55.

Schiller, G., Vescio, R., Freytes, C., Spitzer, G., Sahebi,, F., Lee, M. Wu, S.-H., Cao, J., Lee. J.C., Hong, C.H. Lichtenstein, A., Lill, M., Hall, J., Berenson, R., and Berenson, J. (1995) *Transplantation of CD34+ peripheral blood progenitor cells after high-dose chemotherapy for patients with advanced multiple myelom*a. Blood 86: 390-397.)

The advantage of using HA as an agent to mobilize stem cells for transplantation over the cytokines now used in the art - GM-CSF and G-CSF are numerous. HA has fewer side effects than the cytokines, and appears to act more rapidly, mobilizing a more diverse spectrum of hematopoietic cells, and more of them.

2. HA infusion can be used as a method for supplementary immunotherapy after chemotherapy to mobilize polymorphs and monocytes for front line mechanisms needed in defense against pathogens until recovery from chemotherapy. Currently G-CSF and GM-CSF are used for this purpose, and are administered subcutaneously or intravenously until neutrophil recovery is observed. The advantages to using HA mentioned under paragraph 1 above apply equally here. Treatment of patients with HA as described herein can be substituted for infusion of cytokines

in already existing clinical protocols such as those described in the References below:

(Weaver, C.H., Hazeltonn, B., Birch, R., Palmer, P., Allen, A., Schwwartzberg, L. and West, W. (1995). *An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy*. Blood 86: 3961-3969.

Boiron, J.-M., Marit, G., Faberes, C., Cony-Makhoul, P., Foures, C., Ferrer, A..-M., Cristol, G., Sarrat, A., Girault, D., and Reiffers, J. (1993) *Collection of peripheral blood stem cells in multiple myeloma following single high-dose cyclophosphamide with and without recombinant human granulocyte-macrophage colony-stimulating factor (rh GM-CSF)*. Bone Marrow Transplantation 12: 49-55.

Schiller, G., Vescio, R., Freytes, C., Spitzer, G., Sahebi,, F., Lee, M. Wu, S.-H., Cao, J., Lee. J.C., Hong, C.H. Lichtenstein, A., Lill, M., Hall, J., Berenson, R., and Berenson, J. (1995) *Transplantation of CD34+ peripheral blood progenitor cells after high-dose chemotherapy for patients with advanced multiple myeloma*. Blood 86: 390-397.)

3. HA treatment can also be used as an adjunct to cancer chemotherapy in the following way. In multiple myeloma, other malignancies of the immune system, and metastatic cancer such as breast cancer and small cell lung cancer, malignant cells are/become sequestered or anchored in the bone marrow and/or other tissues. If these malignant cells could be mobilized from the bone marrow or other tissue into the peripherary, they might be more susceptible to chemotherapeutic agents and more effectively killed. A variety of evidence indicates that unanchored cancer cells in suspension have differing susceptibility to a variety of agents

than do anchored cancer cells or cancer cells within an aggregate. Higher drug concentration can be achieved in the blood as compared to the bone marrow, and forcing metastatic migrants into the blood would cause their exposure to this higher dose. Treatment with HA prior to the administration of chemotherapeutic agents is expected to optimize the ability of the chemotherapy to target malignant cells. In this regard, the administration of HA will be given prior to the treatment with chemotherapeutic agents such as the combining of the chemotherapeutic agents with HA as taught in WO 91/04058. Such prior administration will be given in effective amounts (such as 6mg/kg of body weight) preferably at least about 4-24 hours before administration of the chemotherapeutic agent. HA infusion, I believe, will thus facilitate drug-mediated cancer cell kill.

4. HA treatment can also be used to mobilize components of the acquired/adaptive immune response such as T cells and B cells as understood by those skilled in the art. For example, in immunotherapy of AIDS HA can be used as follows. Evidence suggests that CD4+/CD8+ ratios are abnormal mainly in the blood of AIDS patients, but that solid lymphoid tissues such as spleen and lymph node have normal numbers of CD4+ cells. Treatment of patients with HA is expected to mobilize CD4+ T cells from solid lymphoid organs, which would be expected to mediate immune protection to AIDS patients. This will be useful for lymphadenopathy prior to full blown AIDS. Unlike most approaches to treating AIDS, treatment with HA is safe and has no known detrimental side effects. A similar immunodeficiency is frequently exhibited in cancer patients. HA infusion is therefore expected to ameliorate the immunodeficiency. These teachings therefore appear applicable to other conditions involving acquired defects in the adaptive immune response.

5. It is known in the art that proteoglycans and glucosaminoglycans distinguish different sets of mast cells. Treatment with HA, I now believe, mobilizes mast cell progenitors from the bone marrow and peripheral sites

(lung, skin, etc.). This would alter the biodistribution of types of mast cells in the blood and tissue and thus modulate symptoms of allergy and asthma. Infusion with HA is expected to mobilize mast cells from tissue into blood and away from local sites of reaction.

6. HA would also be used to mobilize osteoclasts in order to deplete their number within the bone marrow with the aim of reducing their destructive effect on bone mass in osteoporosis and other bone diseases. Based on the properties of osteoprogenitors, osteoblasts and osteoclasts, it is expected that HA will selectively deplete the osteoclasts from the bone marrow, leaving osteoblasts in situ.

7. Administration of HA causes the rapid appearance of erythroblasts in the peripheral blood (see herein). Infusion of HA will therefore be a useful tool in treating acquired anemias. These anemias include iron deficient anemia, anemia occurring post-surgery, infection–related anemia, insulin–related anemia, low hemoglobin anemias of pregnancy, anemia resulting from blood loss or from poor red blood cell production or destruction. Like erythropoietin, HA mobilizes red blood cells to the circulation.

The invention has other uses as would be understood by persons skilled in the art from the following.

According to another aspect of the invention, a method is provided to mobilize any type of susceptible cell from one tissue to another, as a single agent or before/during other clinical procedures, as taught for hematopoietic and other types of normal or malignant cells by the infusion (use) of effective amounts of HA. These cells include non-hematopoietic normal cells and have the potential during at least one differentiation stage in their life cycle to undergo mobilization/migration in vivo, for example the mobilization of oocytes from the ovary to the fallopian tubes. Clinically, this process is invoked for collection of oocytes to be used for in vitro fertilization. HA will, I believe, improve oocyte release when used in conjunction with other clinical procedures used by those skilled in the art. The invention also provides a method of HA infusion with or without other treatments known to t hose skilled in the art, to improve fertility treatments in vivo.

According to another aspect of the invention a method is provided to mobilize hematopoietic cells before and during harvesting of tissue to be used for organ transplantations by the infusion of effective amounts of HA. The harvested tissue will, I believe, be free of passenger lymphocytes and other hematopoietic and dendritic-type cells, that have been shown to stimulate organ rejection. It also provides a method to use ex vivo HA perfusion to mobilize hematopoietic and dendritic-type cells out of an ex-vivo organ that has already been harvested from the donor. This includes, for example, the in vivo perfusion of tissues in a legally dead organ donor in vivo prior to harvesting of the organ, e.g. heart, liver/lung, kidney or other tissues required for organ transplantation. This also includes HA infusion before and during perfusion ex-vivo that occurs after an organ to be used for transplantation has been harvested from the organ donor prior to grafting it into the recipient host. This method of infusing HA before and during perfusion regimens in vivo and/or ex-vivo will, I believe, substantially improve depletion of donor hematopoietic cells as compared to perfusion solutions without HA as used by those skilled in the art, and thus improve graft survival. This includes a form(s) of HA as taught in the invention including forms of low molecular weight HA (smaller forms).

According to another aspect of the invention, a method is provided using HA infusion to treat host individuals about to receive an organ transplant prior to and during the transplantation procedure by the infusion (use) of effective amounts of HA. This will mobilize any host hematopoietic or dendritic-type cells out of/away from the site of organ transplant. This will delay the ability of host hematopoietic or dendritic-type cells to home to the transplanted organ and force them to remain in the circulation, thus maximizing the effects of subsequent or simultaneous treatment with immunosuppressive agents.

According to another aspect of the invention, a method is provided using HA infusion to mobilize hematopoietic cells and dendritic-type cells away from/out of an organ graft that shows signs of immunologic rejection, as understood by those skilled in the art by the infusion (use) of effective amounts of HA. Infusion of HA with or without immunosuppressive regimens used by those skilled in the art, will stimulate the migration/mobilization out of the threatened organ graft of infiltrating host hematopoietic cells that attack an organ graft. This mobilization, as taught in the invention, will force the graft-infiltrating

hematopoietic and dendritic-type cells into the blood where they are more effectively immunosuppressed by agents used by those skilled in the art.

According to another aspect of the invention, a method is provided to optimize immunosuppressive regimens used by those skilled in the art to dampen or inhibit immune responses, for example in organ or hematopoietic cell transplantation, in autoimmune and autoimmune-like conditions, and in asthma/allergy, or in any condition involving damaging immune reactivity. Such method comprises administration to a patient of an effective amount of HA to optimize the immuno-suppressive regimens used in patients to dampen or inhibit immune responses. Levels of immunosuppressive agents in the blood exceed those in other tissues. Mobilization of rejecting or autoreactive hematopoietic cells, particularly lymphocytes and dendritic-type cells, from the tissues at risk or autoimmunity or rejection into the blood will reduce-halt the immunologic attach and facilitate immunosuppression of the attacking hematopoietic and dendritic-type cells mobilized into the blood by increasing their exposure to immunosuppressive agents.

According to another aspect of the invention, a method to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells by infusing HA before and during the cytoreductive therapy administered prior to an autologous or allogeneic hematopoietic cell transplant in, for example, cancer patients such method comprises administration to a patient of an effective amount of HA to maximize chemotherapeutic kill of hematous poretic and dendritic-type cells in patients benefiting from same. Infused HA will mobilize hematopoietic and dendritic-type cells into the blood where they become more susceptible to the cytoreductive agents used by those skilled in the art, including chemotherapy and/or irradiation regimens, based on evidence that single cells are more vulnerable to these agents than are cells in contact with other cells and microenvironmental factors (i.e. stromal cells and extracellular matrix components) (i.e. in an anchored microenvironmental or as a cellular aggregate).

Embodiments of the invention will now be illustrated with reference to the following Figures in which:

BRIEF DESCRIPTION OF FIGURES

Figures 1-6 depict the results of the flow cytometry technique of cell analysis that identifies cells, in this case, the white blood cells (the red cells were specifically excluded from the analyzed cells) according to their cell

surface characteristics (using known antibodies to detect them) and their sizes, taken from healthy individuals who were administered the form of hyaluronic acid, sodium hyaluronate, at time "0" and from whom blood was drawn at time: 0 (Figure 1); 1 hour after administration of sodium hyaluronate (Figure 2); 4 hours after administration (Figure 3); 12 hours after administration (Figure 4); 24 hours after administration of sodium hyaluronate (Figure 5); and 72 hours after administration (Figure 6). [The blood cells used had been previously purified using centrifugation over Ficoll-Paque™ (commercially available from Pharmacia).]

DETAILED DESCRIPTION OF FIGURES

Thus, Figure 1 (and the other Figures 2-6) provide plots of cell characteristics including granularity (plotted vertically) and forward scatter (FSC-height) relating to size of cells (plotted horizontally). The Plot shows different fractions labeled G_1 to G_4 (corresponding to R_1 to R_4 with R_5 being the entire field) of which G_4 is the one of concern displaying changes in the number of larger granular cells which I have concluded have exited/emigrated the bone marrow. (R_5 is the entire field of analyzed cells.) I have concluded that the presence of the large granular cells is indicative of generalized mobilization that includes stem cells (which are small and therefore have a low light scatter and were not specifically identified in this assay) and other hematopoietic cells.

The cells that fall into the region identified as G4 are those that have high light scatter properties. The high forward scatter indicates large physical size. The high side scatter indicates increased granularity. In normal individuals, peripheral blood mononuclear cells include very few cells in this size fraction, in the range of about 0–3%. Infusion of HA caused a greatly increased number of cells to appear in this size fraction, indicating to me that they were newly emigrated into the blood. Normally, most cells with high light scatter are bone marrow residents. As an example, plasma cells, the cells that are high rate secretors of antibody, are in this size fraction, and appear in the blood only in pathological conditions such as in multiple myeloma. The expression of CD19, a B cell marker present on some plasma cells, and the high scatter indicated to me that HA had mobilized bone marrow plasma cells into the blood of the normal volunteers. The majority of normal plasma cells are bone marrow localized. After HA infusion, the number of cells with high scatter increased dramatically over the 72 hour study period.

It is therefore clear that the percentage of the cells in G_4 relative to all the cells in the field. (R_5) which comprises G_1 (which corresponds to R_1), G_2 (which corresponds to R_2), G_3 (which corresponds to R_3), and G_4 which corresponds to R_4) and the others found in R_5 not previously accounted for are with respect to:

Figure 1 (after 0 hours) → 5% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 2 (after 1 hour) → 9% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 3 (after 4 hours) → 26% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 4 (after 12 hours) → 30% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 5 (after 24 hours) → 9% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 6 (after 72 hours) → 6% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

(Each of Figures 1-6 is accompanied by supporting data and chart plotting. Counts v. FSC-Height)

The data shown is based on examples wherein the amount of sodium hyaluronate equals or exceeds 1.5mg/kg of body weight per patient (for example, 6mg/kg and 12 mg/kg which provide very similar results). Before administering the 6 mg/kg and 12 mg/kg amounts, patients were administered 1.5 mg/kg and 3.0 mg/kg as discussed.

The characteristics of the sodium hyaluronate used with the protocols are set out below:

"A"

TESTS	SPECIFICATIONS	RESULTS
pH	5.0 to 7.0 at 25 degrees C.	6.0
Specific Gravity	0.990 to 1.010 at 25 degrees C.	1.004
Intrinsic Viscosity	4.5 to 11.0 dL/g.	7.07
Molecular Weight	178,000 to 562,000 daltons	319,378 daltons
Sodium Hyaluronate	9.0 to 11.0 mg/mL. Positive	9.9 mg/ML
Assay and Identification		Positive

Another amount may comprise:

	TESTS	SPECIFICATIONS
1.	Description	White or cream odourless powder
2.	Identification (IR Spectrum)	Conforms to Ref. Std. Spectrum
3.	pH (1% solution)	5.0 to 7.0
4.	Loss on Drying	NMT 10%
5.	Residue on Ignition	15.0% to 19.0%
6.	Protein Content	NMT 0.1%
7.	Heavy Metals	NMT 20 ppm
8.	Arsenic	NMT 2 ppm
9.	Residual Solvents	
	a) Formaldehyde	NMT 100 ppm
	b) Acetone	NMT 0.1%
	c) Ethanol	NMT 2.0%
10.	Sodium Hyaluronate Assay (dried basis)	97.0 to 102.0%
11.	Intrinsic Viscosity	10.0 to 14.5 dL/g
12.	Molecular Weight	500,000 to 800,000 daltons
13.	Total Aerobic Microbial Count (USP 23)	NMT 50 microorganisms/g
14.	Escherichia coli (USP 23)	Absent
15.	Yeasts and Moulds (USP 23)	NMT 50 microorganisms/g
16.	Bacterial Endotoxins (LAL) (USP 23)	NMT 0.07 EU/mg

The following protocol was followed for administering the sodium hyaluronate identified under "A" in the preceding pages and the drawing of blood from the patients to whom the sodium hyaluronate was administered.

Four healthy non-smoking female volunteers and four healthy non-smoking male volunteers were given, at different times (at least 7 days between dosages) the following dosages:

(A) 1.5 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(B) 3.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(C) 6.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(D) 12.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(The hyaluronic acid solution was as described herein as "A" in the preceding pages of this specification.)

In each case, a total volume of 250 ml was infused. Therefore, the 1% hyaluronic acid solution as required was diluted with an appropriate volume of 0.9% sodium chloride solution. The infusion was over a period of 120 minutes.

Each of the dosages was administered in an ascending manner (1.5 mg/kg, 3.0 mg/kg, 6.0 mg/kg, and 12.0 mg/kg) to each of the individuals with at least 7 days between doses. The individuals were asked to engage in normal activity for the first four hours after drug administration avoiding both vigorous exertion and complete rest.

Blood samples were drawn from each person at time intervals of 0, 1, 4, 12, 24 and 72 hours after the administration of each of the dosages.

The cells in the drawn samples were analyzed by a known cell analysis technique, termed flow cytometry. Figures 1-6 illustrate the results of analyzing the purified white blood cells in the blood samples taken from the individuals after 0, 1, 4, 12, 24, and 72 hours after administration of 12 mg/kg of body weight of the sodium hyaluronate by intravenous infusion of the individuals, after purification. (Red cells have been excluded from the analysis of these tests.)

Area G_4 displays cells with high light scatter, herein termed large cells, in the blood samples. Cells that have these properties include plasma cells, polymorphonuclear cells (such as granulocytes, neutrophils, and the like) or osteoclasts. Normally, at time, t = 0 hours [at infusion], only small amounts of these cells are present in normal blood (less than 3% of the total of white cell [R_5] population). After administration of 12 mg of sodium hyaluronate/kg of body weight, their presence increases. [Increases were also visible when lower amounts of HA/kg were infused but are not shown.]

After 12mg/kg infusion into one normal individual, blood sample was taken, purified and analyzed with the following results:

time (t) hours	% of cells that are large cells
1	9.47
4	25.88
12	30.03

24	9.10
72	6.80

These large cells, which are detected at relatively high numbers in the blood after HA infusion, I have concluded are emigrants from the bone marrow. Because their scatter properties are identical to those of late stage B cells and plasma cells that arise in malignant conditions (e.g. multiple myeloma), I have also concluded that they include plasma cell migrants from the bone marrow, the major site of plasma cells in the body. Phenotypic characterization of these large cells arising after HA infusion indicates that they express a low density of the B cell marker CD19, also consistent with their identity as plasma cells. If plasma cells are stimulated to migratory behavior by HA infusion, other cell types, in particular stem cells, will also be stimulated into migratory behavior. Thus these large plasma–like cells, I have concluded, are indicators of hematopoietic cell migration from the bone marrow into the blood. HA upregulates HA receptors as well as sending signals to the cell that activate motogenic behavior and ultimately migration out of the marrow into the blood.

Thus, instead of using recombinant GM-CSF with its adverse effects, the individual may now receive a form of hyaluronic acid (without the same side effects). Because of a lack of toxicity, greater amounts than 12mg/kg of body weight may be administered to a patient for the effect. I have also found that an amount of 6mg sodium hyaluronate/kg of body weight administered to an individual has the similar effect as the administration of 12mg sodium hyaluronate/kg of body weight. Lesser amounts than 6mg/kg of administered HA achieved detectable effects on the cell types in the peripheral blood. During this 4 week infusion protocol the earlier doses impacted on the later doses. For example, the 1 mg/kg and 3 mg/kg of patient weight "primes" the patient so that lesser amounts of the form of hyaluronic acid may be suitable to be effective to stimulate the production/release of the hematopoietic cells at subsequent doses.

The Example discussed above has also been specified as follows:

According to the Example, HA (a 1% solution from Streptomyces, of molecular weight 200,000-300,000, with less than 0.1% protein contaminants [Hyal Pharmaceutical Corporation, Mississauga, Ontario] described as "A" previously of the application, was infused intravenously

into each of 6 normal adult volunteers weekly for 4 weeks. The dosage of HA given on each of the 4 weeks was:

Week 1: 1.5 mg of HA per kg of body weight,
Week 2: 3 mg of HA per kg of body weight,
Week 3: 6 mg of HA per kg of body weight,
Week 4: 12 mg of HA per kg of body weight,

The dosage was infused over a period of approximately 2 hours each week.

Each week, blood samples were taken at time zero (immediately before infusion), and at time points 1 hour, 4 hours, 12 hours, 24 hours and 72 hours after infusion was initiated. White blood cells were purified using standard clinical laboratory techniques, and then analyzed for their physical properties (size and granularity) using flow cytometry methods which are well established in the art. Cells were stained with monoclonal antibodies (tagged with a phycoerythrin, which gives orange staining) which are accepted in the art to detect B cells (CD19), T cells (CD4 and CD8) and monocytes (CD14). The ability to bind HA was detected by exposing cells to FITC-labeled HA (which gives green staining). White blood cell types were defined by these properties as is widely accepted in the art of clinical analysis.

Results

The infusion with HA caused the appearance in blood of white blood cell types and numbers not seen in normal blood. The following changes were observed in samples of blood taken after HA infusion.

Increase in polymorphonuclear cells

A subset of large cells with high side scatter (SSC), having the properties of highly granular polymorphonuclear cells increased from about 4% to about 30% of total peripheral blood cells. This increase occurred most markedly on weeks 3 and 4 (the 6 and 12 mg/kg doses), and over the time period from 4 to 12 hours after infusion.

Appearance of erythroblasts

At the 4 hour time point on week 4 (the 12 mg/kg dose), the blood sample had many red cells aggregating on the interface of the Ficoll-Paque™ purification gradient. This does not occur in normal blood, but is frequent in bone marrow and some blood samples taken after chemotherapy for multiple myeloma, and is usually due to the presence of erythroblasts that were increased in number after chemotherapy. I therefore conclude that erythroblasts were mobilized by infusion of HA.

Appearance of plasma cells

At the 72 hour time point, on week 4 (the 12 mg/kg dose), there were large numbers of large granular cells with a dim CD19 (a B cell marker) expression, and high HA binding, the characteristics of plasma cells. Normally, plasma cells are not found in blood, and the majority of plasma cells in normal individuals are located in the bone marrow. This suggests that HA has released plasma cells from the bone marrow. This does not appear to occur for cytokine mobilizations, suggesting that HA mobilizes cellular types not usually mobilized.

Appearance of early stage monocytes

On week 4, at time zero, there are large numbers (20 to 40%) of cells having the scatter properties expected for monocytes. However, use of a monocyte marker did not detect them, and unlike monocytes, they did not bind HA. Since they are present only at time 0, and only on week 4, they are late migrants to the blood resulting from the previous infusion of HA. They appear to be early stage monocytes.

Increase in proportion of small cells

At the 24-72 hour time points for weeks 3 and 4 (the 6 and 12 mg/kg doses), most samples had a marked increase in the proportion of small cells. These include T cells, B cells and probably hematopoietic stem cells.

The unusual populations in the purified white blood cells after HA infusion, I have thus concluded, are cells that have migrated to the blood as a result of exposure to HA. In one example this is due to a competition effect that mediates release from the bone marrow matrix, as well as an activation of cell migration by HA (which is known to be a central player in cell motility). The patterns indicate early (4hr) release/migration of polymorphs and erythroblasts (relatively late stage red cell progenitors which are nucleated), later release of stem cells, small lymphocytes, and plasma cells (24-72 hours) and very late release of monocytoid cells (7 days). The most dramatic changes occur at week 4 and tend to be progressively increasing over the 4 week period. Thus many infusions of HA mobilize more cells, and more types of cells than only one infusion, and higher concentrations mobilize more cells and cell types than lower doses. The mobilization at 4 hours and later correlates with the presence in circulation and in the body of smaller fragments of HA. Digested HA may be a better mobilizing agent than large HA. As little as one exposure to the lowest dose of HA alters purified white blood cell populations indicative of mobilization. However, it happens more reproducibly at

higher doses or after more than one infusion. Close inspection of the data taken at weeks 1 and 2 (after infusion of 1.5 or 3mg/kg concentrations of HA) indicated that at least mobilization of large cells occurred. Inspection of data of untreated normal people and of the time 0 data for the 3 subsequent cycles of treatment indicated that the effects of HA were cumulative as the proportion of high scatter cells gradually increased over the 4 week period.

As many changes can be made to the embodiments without departing from the scope of the invention, it is intended that all material contained herein be interpreted as illustrative of the invention and not in a limiting sense.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE AS FOLLOWS:

1. The use of an effective amount of a form of hyaluronic acid having a molecular weight less than 750,000 daltons and greater than 150,000 daltons selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing the stimulation of hematopoietic cell production, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

2. The use of Claim 1 wherein the hematopoietic cells comprises the cell selected from the group consisting of a ganulocyte, macrophage, CD34+ stem cell, monocyte, erythrocytes, polymorphonuclear cell, osteoblasts, osteoclasts, mast cells, T-cell, B-cell, and platelets.

3. A method of treating an individual suffering from anemia, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons to the individual, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

4. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing the stimulation of cell production/release from the bone marrow and other tissue sites into the blood, the cells being selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, the molecular weight of the form of hyaluronic acid being less than about 750,000 and greater than 150,000 daltons, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

5. A method of treating an individual for enhancing the stimulation of the production/release from the bone marrow and other tissue sites into the blood of cells selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, comprising administering an effective amount of a form of hyaluronic acid selected from the group

consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to an individual the molecular weight of the form of hyaluronic acid being less than 750,000 daltons, wherein the amount of the form of hyaluronic acid is between 10mg to 3000mg.

6. The use of an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons and greater than 150,000 daltons in the manufacture of a pharmaceutical composition for administration to an individual for enhancing the stimulation of cell production/release, from the bone marrow and other tissue sites into the blood, the cells being selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

7. The use of an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for stimulating and activating stromal cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

8. A method of treating an individual for enhancing the stimulation and activation of stromal cells, comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to an individual, the molecular weight of the form of hyaluronic acid being less than 750,000 daltons.

9. The use of an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid pharmaceutically acceptable salts having a molecular weight less than 750,000 daltons and greater than 150,000 daltons in the manufacture of a pharmaceutical composition for administration to an individual for enhancing the stimulation and activation of stromal cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

10. The use of an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for releasing cancer cells from bone marrow and other tissues into the blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

11. A method of treating an individual for releasing cancer cells from bone marrow and other tissues into the blood comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to an individual, the molecular weight of the form of hyaluronic acid being less than 750,000 daltons.

12. The use of an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid pharmaceutically acceptable salts having a molecular weight less than 750,000 daltons and greater than 150,000 daltons in the manufacture of a pharmaceutical composition for administration to an individual for releasing cancer cells from bone marrow and other tissues into the blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

13. The use of Claim 1, 2, 4, 6, 7, 9, 10 or 12 wherein the form of hyaluronic acid comprises at least about 1.5mg/kg of individual body weight to whom the form of hyaluronic acid is administered.

14. The use of Claim 1, 2, 4, 6, 7, 9, 10 or 12 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount.

15. The method of Claim 3, 5, 8 or 11 wherein the form of hyaluronic acid comprises at least about 1.5mg/kg of individual body weight to whom the form of hyaluronic acid is administered.

16. The method of Claim 3, 5, 8 or 11 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount.

17. The use of Claim 13 wherein the form of hyaluronic acid is at least about 12 mg/kg.

18. The method of Claim 15 wherein the form of hyaluronic acid is at least about 12 mg/kg of patient body weight.

19. The use of Claim 13 or 14 wherein the form of hyaluronic acid is sodium hyaluronate.

20. The method of Claim 15 or 16 wherein the form of hyaluronic acid is sodium hyaluronate.

21. The use of Claim 19 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

22. The method of Claim 20 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

23. A method of treatment for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for enhancing, stimulating and releasing hematopoietic cells and dendritic-type cells from the bone marrow and other tissues into the blood.

24. A method of treatment for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for stimulating and activating stromal cells.

25. A method of treatment for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for

releasing cancer cells from the bone marrow and other tissues into the blood.

26. The method of Claim 23, 24 or 25 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

27. The method of Claim 23, 24 or 25 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount.

28. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons and greater than 150,000 daltons for the manufacture of pharmaceutical composition for administration to a human for stimulating and releasing hematopoietic cells and dendritic-type cells from the bone marrow and other tissues into the blood.

29. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for the manufacture of pharmaceutical composition for administration to a human for stimulating and activating stromal cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

30. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for releasing cancer cells from the bone marrow and other tissues into the blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg having a molecular weight of greater than 150,000 and less than 750,000 daltons.

31. The use of Claim 28, 29 or 30 wherein the form of hyaluronic acid is sodium hyaluronate.

32. The use of Claim 31 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

33. The use of Claim 28, 29, 30, 31 or 32 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

34. The use of Claim 28, 29, 30, 31 or 32 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional effective dosage amount for stimulating the cell production/release from the bone marrow.

35. The use of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for stimulating the production/release of hematopoietic cells and dendritic-type cells from the bone marrow and other tissues into the blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

36. The use of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons for releasing cancer cells from the bone marrow and other tissues into the blood.

37. The use of Claim 35 or 36 wherein the form of hyaluronic acid is sodium hyaluronate.

38. The use of Claim 37 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

39. The use of Claim 34, 35, 36, 37 or 38 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 1.5mg/kg of body weight to whom the form of hyaluronic acid is administered.

40. The use of Claim 34, 35, 36, 37 or 38 wherein the form of hyaluronic acid comprises at least two dosages, a priming dosage amount and an additional dosage amount.

41. A method of treating a patient for enhancing the stimulation of the production/release from the bone marrow and other tissues of cells selected from at least one of the group consisting of hematopoietic cells and dendritic-type cells, comprising administering a plurality of amounts of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient at predetermined intervals, at least one of such dosages being in an amount suitable to stimulate the production/release of the cells from the bone marrow and other tissues into the blood.

42. The method of Claim 41 wherein the interval between dosages is a week.

43. The method of Claim 41 or 42 wherein at least one of the amounts is a priming dosage for the patient.

44. The method of Claim 41, 42 or 43 wherein the form of hyaluronic acid is sodium hyaluronate.

45. The method of Claim 44 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

46. The method of Claim 41, 42, 43, 44 or 45 wherein one of the amounts is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

47. The method of Claim 41, 42, 43, 44, 45 or 46 wherein one of the dosages is a priming dosage in the amount of less than about 3 mg/kg of patient body weight.

48. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than

150,000 daltons for mobilizing hematopoietic cells from the bone marrow and other tissues in a human into the blood of the human, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

49. A method of treating a patient for mobilizing hematopoietic cells from bone marrow and other tissues in a human into the blood of the human, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient.

50. A method of treating a patient for mobilizing stem cells from bone marrow in a human into the circulation system of the human, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to the patient.

51. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for generating stem cells for transplantation, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

52. A method of generating stem cells for transplantation comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons to an individual and subsequently harvesting the cells to be transplanted from the peripheral blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

53. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for treating immunosuppression caused by chemotherapy,

wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

54. A method of treating a patient for immunosuppression caused by chemotherapy comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient who has undergone chemotherapy.

55. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for treating immunosuppression in a patient caused by AIDS, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

56. A method of a treating a patient for immunosuppression caused by AIDS comprising administering effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient who has AIDS.

57. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for treating cancer, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

58. A method of treating a patient for cancer comprising administering effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient followed by administration of a suitable effective amount of chemotherapeutic agent after about 4 hours.

59. The method of Claim 23 wherein the hematopoietic cells are mast cell progenitors.

60. The method of Claim 59 wherein the treatment is to modulate symptoms of allergy or asthma.

61. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 and greater than 150,000 daltons for increasing the level of red cells in the blood, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

62. A method of increasing the level of red cells in the blood of a patient by administering forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than about 750,000 daltons to the patient.

63. The use of Claim 51, 53, 55, 57 or 61 wherein the form of hyaluronic acid is sodium hyaluronate.

64. The method of Claim 49, 50, 52, 54, 56, 58, 59, 60 or 62 wherein the form of hyaluronic acid is sodium hyaluronate.

65. The use of Claim 63 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

66. The method of Claim 64 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

67. The use of Claim 65 wherein the amount of the form of hyaluronic acid is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

68. The use of Claim 63 wherein the dosage is a priming dosage in the amount of less than about 3mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

69. The method of Claim 64 wherein the amount of the form of hyaluronic acid is at least about 6mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

70. The method of claim 64 wherein the method of treatment includes the administration of a plurality of dosages of the form of hyaluronic acid including at least one priming dosage in the amount of the form of hyaluronic acid less than about 3 mg/kg of patient body weight.

71. A method to mobilize any type of susceptible cell from one tissue to another, as a single agent or before/during other clinical procedures, as taught for hematopoietic and other types of normal or malignant cells, the method comprising administering an effective amount of a form of hyaluronic acid to a patient who will benefit therefrom wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

72. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to mobilize any type of susceptible cell from one tissue to another, as a single agent or before/during clinical procedures as taught for hematopoietic and other types of normal or malignant cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

73. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to mobilize any type of susceptible cell from one tissue to another, as a single agent or before/during clinical procedures as taught for hematopoietic and other types of normal or malignant cells, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

74. A method to mobilize hematopoietic cells before and during harvesting of tissue to be used for organ transplantations by the infusion of effective amounts of hyaluronic acid to a patient wherein the form of

hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

75. A method of using ex-vivo hyaluronic acid perfusion to mobilize hematopoietic and dentritic-type cells out of an ex-vivo organ that has already been harvested from the donor by the infusion of an effective amount of hyaluronic acid to a patient wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

76. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to mobilize hematopoietic cells before and during harvesting of tissue to be used for organ transplantations, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

77. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons and greater than 150,000 daltons to mobilize hematopoietic cells before and during harvesting of tissue to be used for organ transplantations, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

78. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to mobilize hematopoietic and dentritic-type cells out of an ex-vivo organ that has already been harvested from the donor, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

79. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to mobilize hematopoietic and dentritic-type cells out of an ex-vivo organ that has

already been harvested from the donor, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

80. A method using hyaluronic acid infusion to treat host individuals about to receive an organ transplant prior to and during the transplantation procedure by the infusion of an effective amount of hyaluronic acid to a patient wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

81. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to treat host individuals about to receive an organ transplant prior to and during the transplantation procedure, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

82. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to treat host individuals about to receive an organ transplant prior to and during the transplantation procedure, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

83. A method using hyaluronic acid infusion to mobilize hematopoietic cells and dendritic-type cells away from/out of an organ graft that shows signs of immunologic rejection by the infusion of an effective amount of hyaluronic acid to a patient wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

84. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to mobilize hematopoietic cells and dendritic-type cells away from/out of an organ graft that shows signs of

immunologic rejection, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

85. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to mobilize hematopoietic cells and dendritic-type cells away from/out of an organ graft that shows signs of immunologic rejection, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

86. A method to optimize immunosuppressive regimens to dampen or inhibit immune responses, for example in organ or hematopoietic cell transplantation, in autoimmune and autoimmune-like conditions, and in asthma/allergy, or in any condition involving damaging immune reactivity such method comprises administration to a patient of an effective amount of hyaluronic acid to optimize the immunosuppressive regimens used in patient to dampen or inhibit immune responses wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

87. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to optimize the immunosuppressive regimens used in patient to dampen or inhibit immune responses, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

88. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to optimize the immunosuppressive regimens used in patient to dampen or inhibit immune responses, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 12mg/kg.

89. A method to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells by infusing HA before and during the cytoreductive

therapy administered prior to an autologous or allogeneic hematopoietic cell transplant in, for example, cancer patients such method comprises administration to a patient of an effective amount of hyaluronic acid to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells in patients benefiting from same wherein the form of hyaluronic acid is selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 daltons.

90. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons for the manufacture of a pharmaceutical composition to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells in patients benefiting from same, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

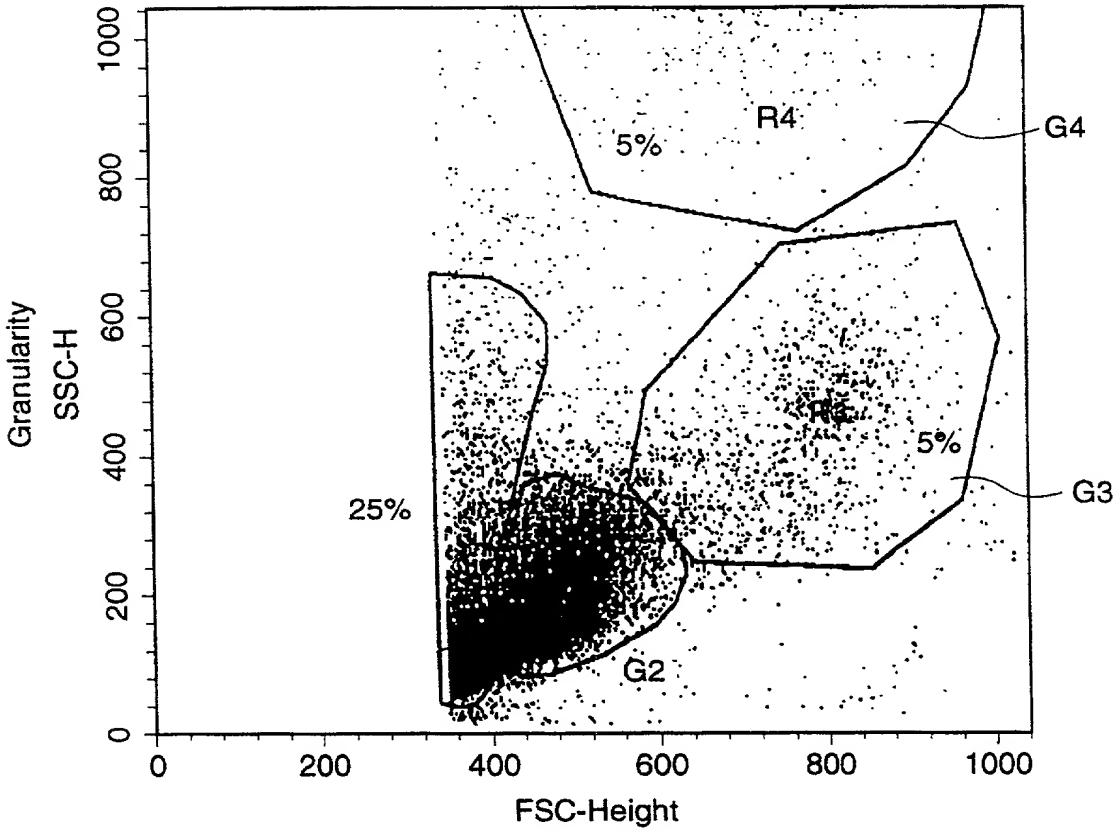
91. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof having a molecular weight less than 750,000 and greater than 150,000 daltons to maximize chemotherapeutic kill of hematopoietic and dendritic-type cells in patients benefiting from same, wherein the amount of the form of hyaluronic acid is between 1.5mg/kg to 3000mg/70kg person.

92. The method of Claim 71, 74, 75, 80, 83, 85 or 89 wherein the form of hyaluronic acid is sodium hyaluronate.

93. The use of Claim 72, 73, 75, 77, 78, 79, 81, 82, 83, 85, 87, 88, 90 or 91 wherein the form of hyaluronic acid is sodium hyaluronate.

94. The use of Claim 93 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

95. The method of Claim 92 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.



File: N24.0HR.052
Sample ID: HAFITC CD4PE
Gated Events: 20235
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 20235
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	5091	25.16	25.16	362.40	362.02	143.68	122.43	1,2
R2	11752	58.08	58.08	460.67	458.78	183.54	174.62	1,2
R3	1046	5.17	5.17	733.07	727.10	415.72	403.53	1,2
R4	957	4.73	4.73	703.43	697.21	1002.78	1001.41	1,2
R5	19853	98.11	98.11	461.99	451.20	230.98	182.21	1,0

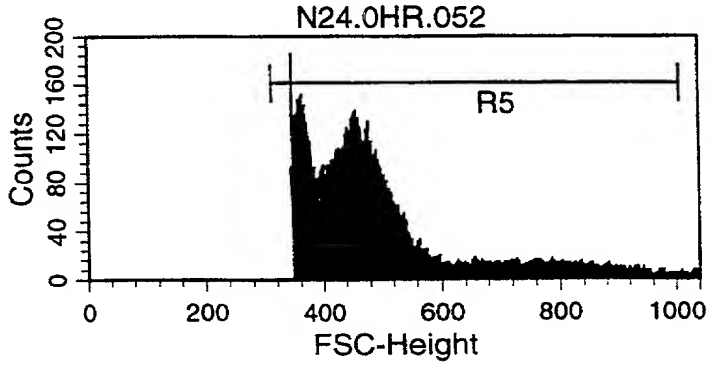
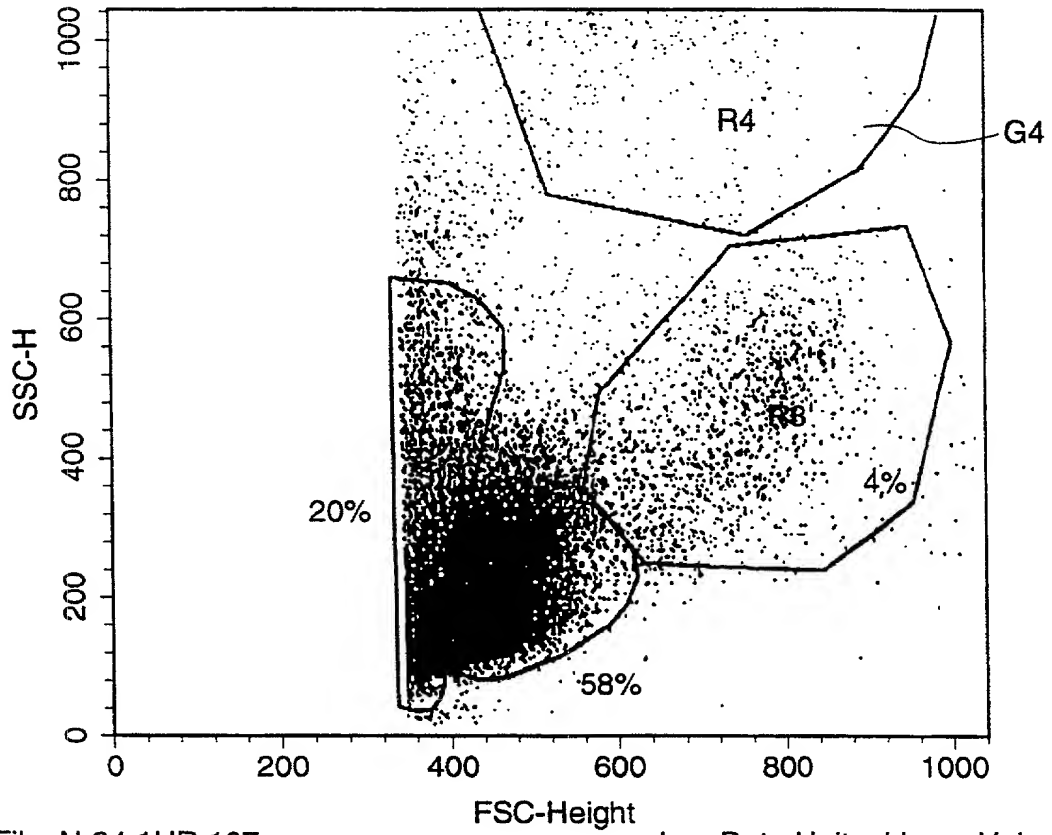


Fig 1



File: N-24 1HR.107
Sample ID: HAFITC CD4PE
Gated Events: 29655
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 29655
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	5929	19.99	19.99	371.93	371.42	229.53	198.56	1,2
R2	17181	57.94	57.94	449.78	448.35	194.16	184.07	1,2
R3	1316	4.44	4.44	721.76	716.46	435.30	422.11	1,2
R4	2809	9.47	9.47	694.70	687.82	1009.13	1007.87	1,2
R5	29255	98.65	98.65	470.17	459.19	304.03	236.96	1,0

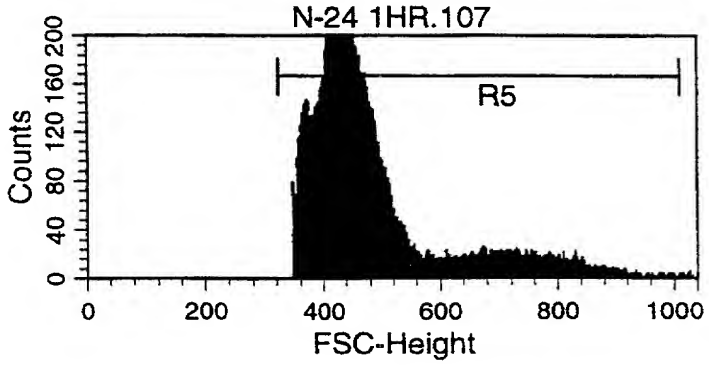
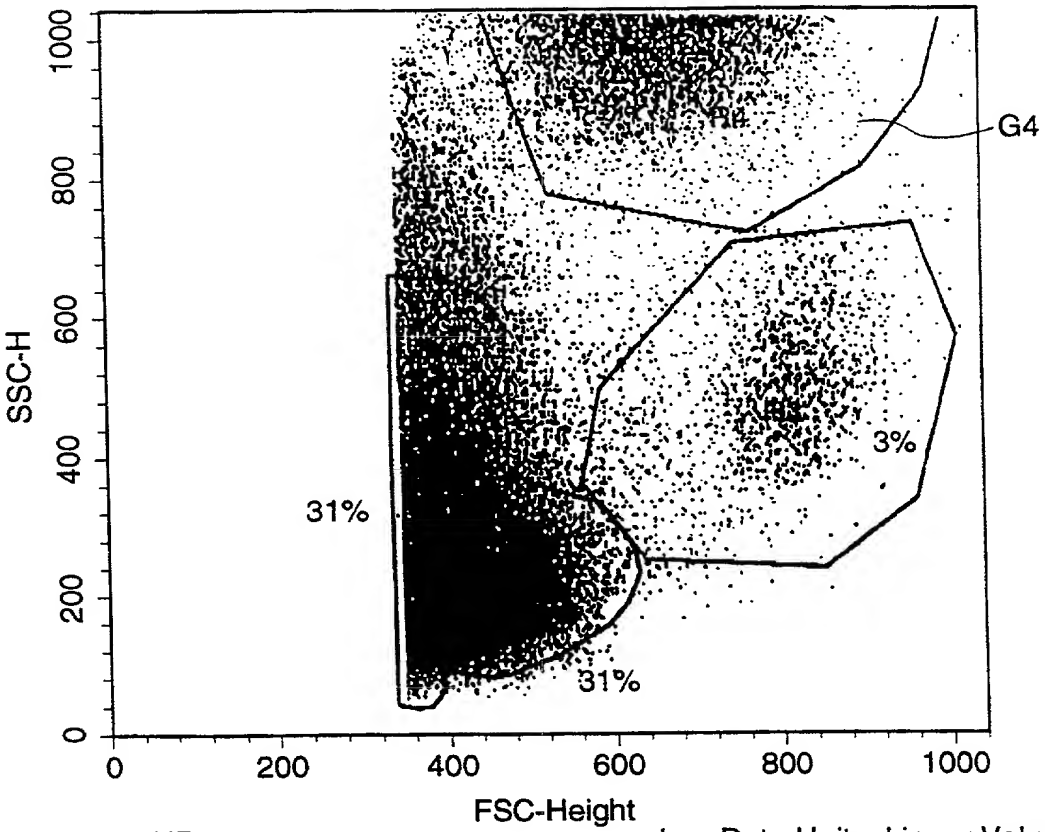


Fig 2



File: N-24 4HR.161
Sample ID: HAFITC CD4PE
Gated Events: 50000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 50000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	15374	30.75	30.75	374.55	373.82	302.56	271.09	1,2
R2	15359	30.72	30.72	452.13	450.52	200.91	192.99	1,2
R3	1509	3.02	3.02	764.50	759.01	477.03	465.53	1,2
R4	12940	25.88	25.88	688.23	679.12	997.82	996.43	1,2
R5	49787	99.57	99.57	498.00	479.12	481.11	367.27	1,0

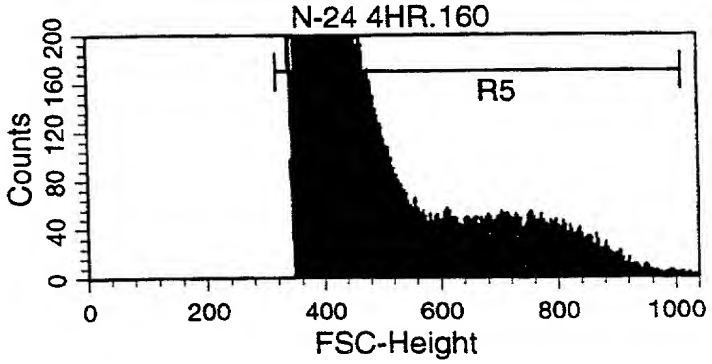
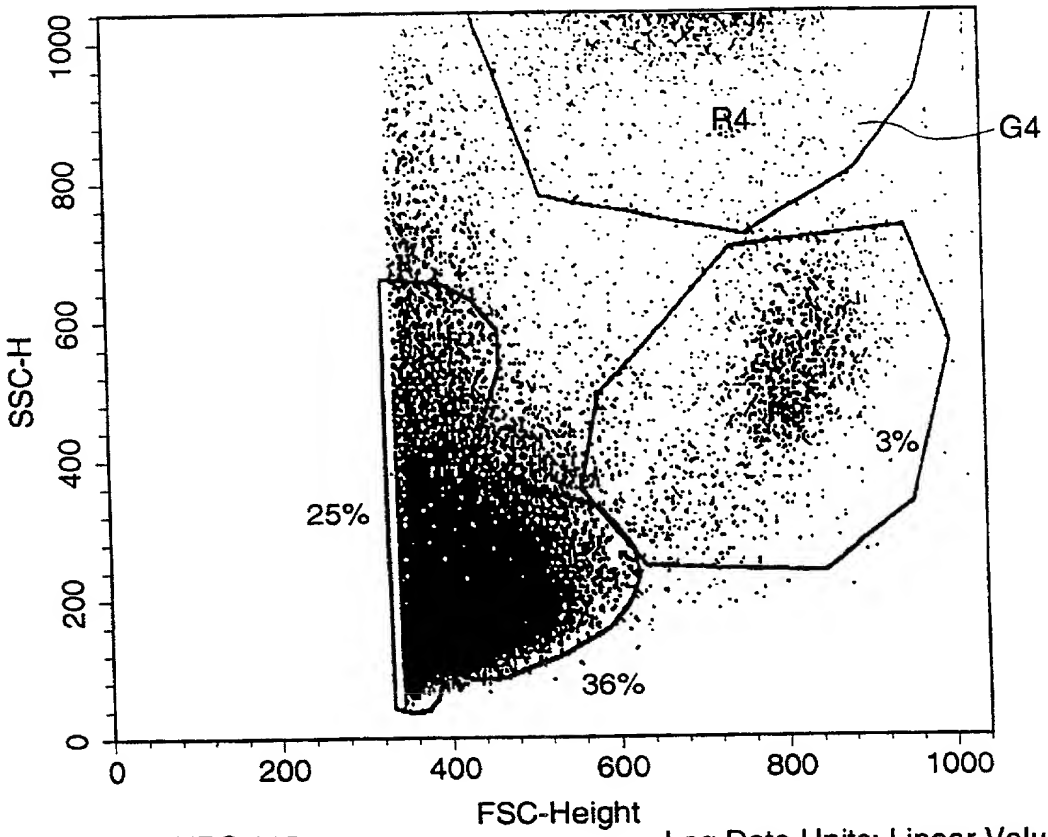


Fig 3



File: N-24 12HRS.215
Sample ID: HAFITC CD4PE
Gated Events: 50000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 50000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	11868	23.74	23.74	369.87	369.33	244.22	211.18	1,2
R2	18416	36.83	36.83	448.85	447.39	184.25	177.16	1,2
R3	1631	3.26	3.26	770.24	765.15	485.71	472.28	1,2
R4	15013	30.03	30.03	727.15	720.73	1017.53	1017.07	1,2
R5	49637	99.27	99.27	524.97	502.11	476.63	337.95	1,0

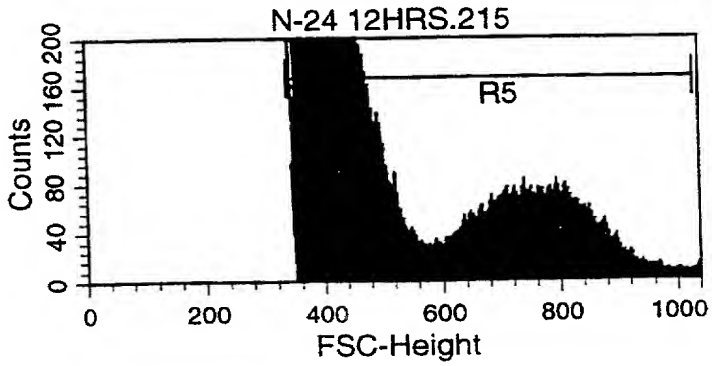
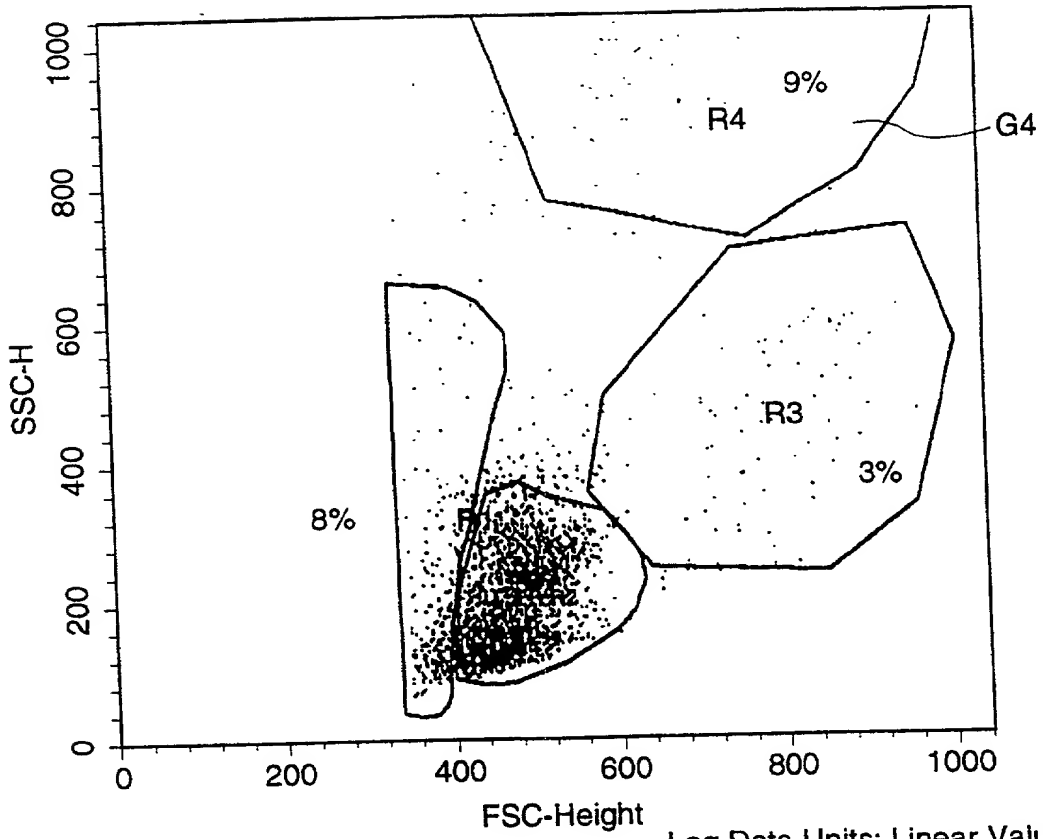


Fig 4



File: N-24 24HRS.271
Sample ID: HAFITC CD4PE
Gated Events: 3000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 3000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	241	8.03	8.03	372.93	372.39	200.17	172.29	1,2
R2	2227	74.23	74.23	463.11	461.59	190.64	180.25	1,2
R3	91	3.03	3.03	736.37	729.14	428.80	415.01	1,2
R4	273	9.10	9.10	675.01	668.46	997.55	996.15	1,2
R5	2993	99.77	99.77	484.34	476.32	284.63	223.45	1,0

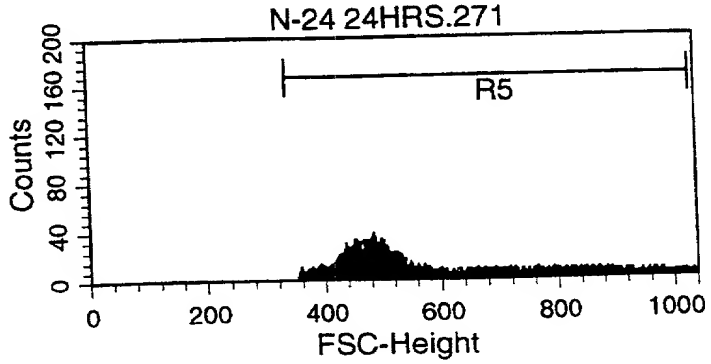
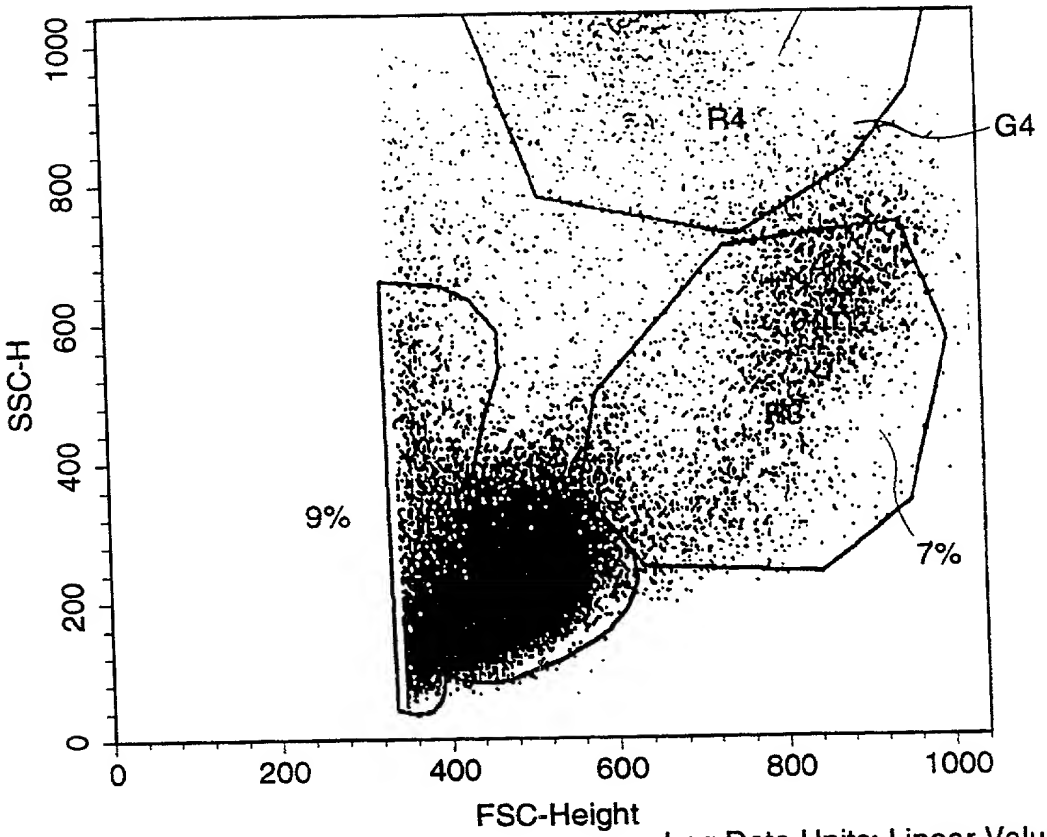


Fig 5



File: N-24 72HRS.326
Sample ID: HAFITC CD4PE
Gated Events: 50000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 50000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	4631	9.26	9.26	371.76	371.26	219.42	192.19	1,2
R2	36587	73.17	73.17	468.81	467.14	205.82	199.29	1,2
R3	2911	5.82	5.82	776.01	768.40	501.23	482.26	1,2
R4	3399	6.80	6.80	694.12	687.24	982.82	979.60	1,2
R5	49829	99.66	99.66	496.29	485.45	289.31	240.51	1,0

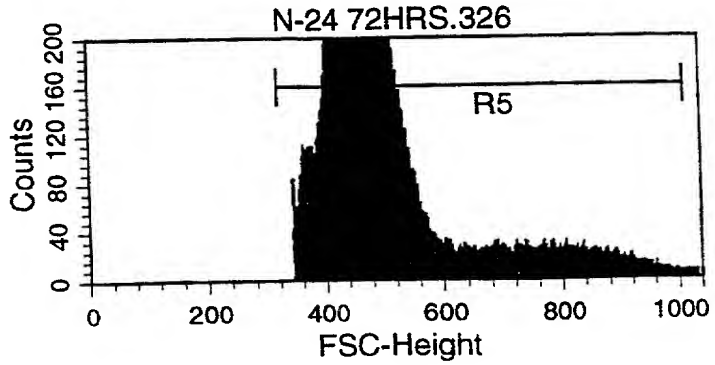


Fig 6

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	PC-1459
First Named Inventor	Linda May Pilarski
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Methods For Cell Mobilization Using In Vivo Treatment With Hyaluronan (HA)

(Title of the invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 03/12/97 as United States Application Number or PCT International Application Number PCT/CA97/00172 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
60/013,401	US	03/14/96	☐	☐	☒
2,173,272	CA	04/02/96	☐	☐	☒
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number → Place Customer Number Bar Code Label here
OR
☐ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Ivor M. Hughes	27,759		
Neil H. Hughes	33,636		
Marcelo K. Sarkis	37,015		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] OR ☐ Correspondence address below

Name	HUGHES, ETIGSON						
Address	175 Commerce Valley Drive West						
Address	Suite 200						
City	Thornhill			State	Ontario	ZIP	L3T 7P6
Country	CANADA	Telephone	(905) 771-6414			Fax	(905) 771-6420

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
~~Linda~~ May	Pilarski

Inventor's Signature	Linda M. Pilarski					Date	28 Dec 98
Residence: City	Stony Plain	State	Alberta	Country	CANADA	Citizenship	CA
Post Office Address	Box 63, Site 3, R.R. #4						
Post Office Address							
City	Stony Plain	State	Alberta	ZIP	T7Z 1X4	Country	CANADA

☐ Additional inventors are being named on the ___ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

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DECLARATION — Supplemental Priority Data Sheet

Additional foreign applications:

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
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			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

Additional provisional applications:

Application Number	Filing Date (MM/DD/YYYY)

Additional U.S. applications:

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

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DECLARATION	REGISTERED PRACTITIONER INFORMATION (Supplemental Sheet)

Name	Registration Number	Name	Registration Number

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